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**PROCEEDINGS**  
OF THE  
**NATIONAL ACADEMY**  
**OF SCIENCES**  
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## ON CERTAIN SEQUENCES OF FUNCTIONS

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A recent article<sup>1</sup> treated the characterization of functions which are odd, periodic of period  $2\pi$  and such that  $\{f(nx)\}$  is orthonormal. We shall indicate here certain new results and refinements which now give a fairly well-integrated picture. Using methods suggested by the primary problem, it has been possible to develop criteria for completeness of certain sets of functions. Full details of the proofs will be published elsewhere.

We mention our main conventions. We use  $L_2(0; -\pi, \pi)$  to designate odd functions in  $L_2(-\pi, \pi)$ . A subsequence of the positive integers has a base  $\{p_j | p_j \text{ a prime}\}$  if each member of the sequence is a product of powers of the elements of the base. The function  $f(x)$ , the sequence of its Fourier sine coefficients  $\{a_n\}$  and the Dirichlet series  $\phi(z) = \sum a_n n^{-z}$  formed with the coefficients are said to be *associated*. The classes  $K$ ,  $K'$  and  $K^u$  refer, respectively, to *solutions* for which  $\{a_n\} \in l_2$ ,  $\{a_n\} \in l_1$  or  $\{a_n\} \in U$ , i.e.,  $\sum a_n n^{-u}$  is uniformly convergent. The same class symbols designate the associated functions and Dirichlet series. The term *solution* is used to indicate satisfaction of  $[f(mx), f(nx)] = \delta_{n,m}$ . A quasi elementary solution is defined by

$$\phi(z) = M^{-z}(1 + \sum_{j=1}^N h_j n_j^z) / (1 + \sum_{j=1}^N h_j n_j^{-z})$$

where  $\{h_j\}$  is a finite real sequence,  $\{n_j\}$  is an ascending sequence of integers  $\geq 2$ ,  $M$  is proportional to the L.C.M. of the  $\{n_j\}$  and the denominator does not vanish for  $R(z) \geq 0$ . It is easy to show that a quasi elementary solution is a solution.

We first remark that practically all the theorems<sup>1</sup> proved for the class  $K'$  remain valid for the class  $K^u$ . The reason for this is essentially that the Dirichlet series represents an almost periodic function for  $R(z) = 0$  and the combination properties of such functions prove sufficient to supplant the more restrictive requirement of absolute convergence. Thus for instance:



*Necessary and sufficient conditions that  $\phi(z) \in K^v$  are: (a)  $\phi(z)$  is meromorphic, (b)  $\phi(z)$  admits a Dirichlet expansion converging uniformly for  $R(z) \geq 0$ , (c)  $\phi(z)\phi(-z) = 1$ .*

It is easy to show a little more.

*If  $\phi(z) \in K^v$  then the uniform convergence abscissa of the Dirichlet series is to the left of  $R(z) = 0$ .*

Some of the earlier uniqueness theorems<sup>1</sup> can be sharpened considerably. Thus the class  $K'$  can be replaced by  $K$ , and as comparison will show, other generalizations are involved as well, below. New methods of proof are required and mapping theorems play a central rôle now. Thus as generalizations of theorem 10.7 and 10.3 we have:

*If  $\phi(z) \in K$  and (a)  $\phi(z)$  is uniformly continuous in  $R(z) \geq 0$ , (b)  $|\phi(it)| = 1$ , (c)  $\phi(z)$  has no zeros in  $R(z) > 0$ , (d)  $\phi(z)$  has a non-vanishing constant term in its Dirichlet expansion, then  $\phi(z) = \pm 1$ .*

*If  $\{a_n\} \in K$  then  $\{a_n n^r / C\}$ ,  $r > 0$ , cannot be in  $K$  unless  $C = N^r$ ,  $N$  a positive integer, and then  $a_n = \delta_{n, N}$ .*

We may show also,

*If  $\{a_n\} \in K$ ,  $a_1 \neq 0$ , then  $\{na_n / C | n = 2, 3, \dots\}$  is never a member of  $K$ .*

*If  $\{a_n'\}$  and  $\{a_n''\}$  are both non null sequences in  $U$ , then the relations  $[f_1(nx), f_2(mx)] = 0$ ,  $n, m = 1, 2, \dots$  are incompatible.*

The proof involves a simple category argument.

One of our key results is the following.

*If  $\phi(z) \in K^v$  has a finite base, then  $\phi(z)$  is a quasi elementary solution.*

The general idea of the demonstration is to associate a function of several complex variables with the Dirichlet series.

In this connection we cite also the following result:

*If  $\phi(z) \in K'$  has the base  $\{h_n\}$  then replacing  $h_n$  by  $h_n'$  where the  $h_n'$ 's are not necessarily distinct, yields a function also in  $K'$ .*

In particular, every reduction to a finite base must therefore yield a quasi elementary solution. This and other internal evidence suggests that if  $\phi(z) \in K'$  then  $\phi(z)$  is a quasi elementary solution and hence has a finite base. The writer has not yet succeeded in settling whether or not this conjecture is true.

Constructive examples of functions,  $\phi(z)$ , in  $K$  may be exhibited for which  $R(z) = 0$  is a natural boundary. This shows at once that the relation  $\phi(z)\phi(-z) = 1$  valid for solutions in  $K^v$  is not valid for general solutions in  $K$ . The next result is therefore of special interest.

*Necessary and sufficient conditions in order that  $\phi(z) \in K$  are that for  $R(z) > 0$ : (a)  $|\phi(z)| \leq 1$ , (b)  $\phi(z)$  has a uniformly convergent Dirichlet series and (c)  $\sum (a_n)^2 = 1$ .*

A Fatou theorem argument shows that if  $\phi(z) \in K$  then  $L_t \rightarrow_{t \rightarrow \infty} \phi(s + it)$  exists for almost all  $t$ . The limit function is written  $\phi(it)$ . (In view of our preceding remark, it is clear that  $\phi(it)$  is in general not the sum function

of the series  $\sum a_n n^{-s}$ .) Moreover  $\phi(z)/(1/2 - z)$  converges to  $\phi(it)/(1/2 - it)$  in the norm topology of  $L_2(-\infty, \infty)$  as  $s \rightarrow 0+$ . If  $\phi(z)$  has a single element base or  $\phi(z) \in K'$  then  $|\phi(it)| = 1$  a.e.

There is a close relationship between our problem and some phases of the Watson theory of General Transforms.<sup>2</sup> Thus let  $H(\lambda) = \sum_{n>1/\lambda} a_n n^{-1/2}$ .

If  $\phi(z) \in K$  and if (a) the function  $\phi(it)$  defined in the limit sense has its modulus 1 a.e., then  $H(\lambda\rho)/\rho$  is a self reciprocal kernel in the Watson sense and defines a unitary transformation in  $L_2(0, \infty)$ .

If  $H(\lambda)$  satisfies  $W$ :

$$\int_0^{\max(k^{-1}, l^{-1})} \frac{H(\lambda k)H(\lambda l)}{\lambda^2} d\lambda = \min(k, l)(1 - (\phi(1/2))^2)$$

then  $\sum a_n n^{-s}$  is convergent for  $R(z) > 0$ ,  $|\phi(s + it)| = O(|t|^{1/2})$ ,  $s > 0$ ,  $L_{T \rightarrow \infty} \frac{1}{2T} \int_{-T}^T |\phi(s + it)|^2 dt$  exists for  $s > 0$  and  $(|\phi(z)|^2/|1/2 - z|^{2\alpha}) \in L_1(-\infty, \infty)$  for  $\alpha > 1/2$ ,  $0 < s < 1/2$ .

These partial inclusions become equivalences in particular cases. Thus:

If  $\{a_n\} \in l_1$  or  $\{a_n\}$  has a one-element base, then a necessary and sufficient condition that  $\phi(z) \in K$  is that  $H(\lambda)$  satisfies  $W$ .

We may remark in this connection that the theory of sequences  $\{\alpha_N\}$  satisfying  $\sum_{N=0}^{\infty} \alpha_N \alpha_{N+k} = \delta_{0,k}$  may be shown to be isomorphic with that of  $\{a_n\} \in K$  under the condition of a one-element base. The isomorphism implies a 1-1 mapping of corresponding sequences and a topological agreement in  $l_2$ .

An interesting viewpoint is afforded by the following result. Let  $\psi_N(z)$  denote the first  $N$  terms in the Dirichlet expansion of  $\psi(z)$ .

If  $B$  is the class of functions  $\psi(z)$  such that for  $R(z) > 0$  the Dirichlet series converges uniformly and  $|\psi(z)| \leq 1$ , then the function  $\psi(z)$  for which  $\psi_N(z)$ ,  $N$  fixed, takes on a maximum value is a quasi elementary solution.

This is a simple consequence of a result of Landau, and indeed the function  $\psi(z)$  is unique up to change of base. The interest of the theorem lies in the *maximum* property. Whether it extends to more general bases is an open question.

We now turn to some completeness problems. As a preliminary we remark that following Szasz' idea of associating the completeness problem with that of the zeros of certain integrals, we may gain a wide variety of theorems on  $\{f(nx)\}$  where  $f(x + iy)$  is analytic. For instance, using the theorem of Carlson we can easily show

If (a)  $f(0) = 0$ , (b)  $f(w)$ ,  $w = x + iy$ , is a regular analytic function for  $x \geq 0$ , (c)  $f'(0) \neq 0$ ,  $l = 1, 2, \dots$ , (d)  $|f(w)| = O(\exp k|w|)$ ,  $k < 1/2$ , for  $x \geq 0$  then  $f(nx)$  is complete in  $L_2(0, 2\pi)$ .

With each theorem of Carlson type we can associate analogous completeness theorems. We now turn to the main ideas of our work in this field. We write  $(i, j)$  for the L.C.M. of the integers  $i$  and  $j$  and  $B_{i,j}$  for  $\sum_{k=1}^{\infty} b_{ik} b_{jk}$ .

We define a minimizing sequence  $\{C_n^M | n = 1, \dots, M\}$  in the obvious way for a function  $F(x) \in L_2(E)$  and a given orthonormal sequence  $\{g_n(x) | g_n(x) \in L_2(E)\}$ . In the case

$$f(x) \sim \sin x + \sum_{n=2}^{\infty} b_n \sin nx \quad (A)$$

it is easy to show

*The sequence  $\{f(nx)\}$  is minimal and if  $\{C_n^M | n = 1, \dots, M\}$  is minimizing for  $F(x) = \sin x$  then*

$$\|\sin x - \sum_{n=1}^M C_n^M f(nx)\|^2 = |1 - C_1^M|.$$

The term *minimal* implies  $f(Nx)$  is not in the closed linear extension of  $\{f(nx) | n \neq N\}$  for any  $N$ .

*If  $f(x)$  is of the form (A) where  $\{b_n | n = 2, \dots\} \in l_2$  then a sufficient condition that  $f(nx)$  be complete in  $L_2(0; -\pi, \pi)$  is that  $\sum_{(i,j)=1} B_{i,j} |B_{1,j}| < 1$ .*

A more general result follows which involves a less perspicuous condition, however.

*If  $\{g_n(x) | n = 1, 2, \dots\}$  is a complete orthonormal set in  $L_2(E)$  and  $\{h_n(x) | h_n(x) \in L_2(E)\}$  is such that the Grammian of  $\{h_n(x) - g_n(x) | n = 1, 2, \dots\}$  has a bound inferior to 1 then  $\{h_n(x)\}$  is complete in  $L_2(E)$ . In particular the completeness follows from  $\sup_{1 \leq j < \infty} \sum_{i=1}^{\infty} [h_i(x) - g_i(x), h_j(x) - g_j(x)] \geq \theta < 1$ .*

If  $f(x)$  is defined by (A) then choosing  $F(x)$  as  $\sin x$  the minimizing sequences converge termwise to  $\{d_n | n = 1, 2, \dots\}$ . It is readily shown that  $\{d_n\}$  is defined uniquely by the requirement that  $\sin x - \sum_{n=1}^M d_n f(nx)$  be orthogonal to  $\sin jx$ ,  $j = 1, \dots, M$ . It may be shown that the Paley-Wiener theorem<sup>3</sup> entails in this case that

$$L_{N \rightarrow \infty} \|\sin x - \sum_{n=1}^M d_n f(nx)\| = 0. \quad (P)$$

Moreover  $\{d_n\} \in l_2$ .

The next results comprehend situations for which the Paley-Wiener theory does not apply. Here it is possible that  $\{\alpha_n\} \in l_2$  and the relation in  $P$  may not be valid.

*If  $f(x) = \sin x - \sum_{n=2}^M b_n \sin nx$ ,  $b_j b_M \neq 0$ , for some  $j$ ,  $j \neq M$ , then a sufficient condition for completeness is that*

$$\sum_{(i,j)=1} B_{i,j} \cos t(\log i - \log j) \leq 1, \quad -\infty < t < \infty. \quad (B)$$

We remark that the requirement  $|b_j b_M| \neq 0$  is essential. The strength

of the theorem comes from the case that equality is admitted in the main condition (B).

The example

$$f(x) = \sin x - \frac{1}{2}(\sin 2x + \sin 4x)$$

is easily shown to give  $\{d_n\} \in l_2$ . Moreover  $\sum_{n=1}^N d_n f(nx)$  does not converge. Nevertheless  $\{f(nx)\}$  is complete in  $L_2(0; -\pi, \pi)$ .

If  $M = \infty$  in the previous theorem, the completeness assertion is still true provided we weaken (B) by replacing the right hand side by " $< 1$  almost everywhere." If we use the stronger restriction " $\leq \theta$ ,  $0 < \theta < 1$ ," then we can guarantee  $\{d_n\} \in l_2$  and Eq. (P) holds. Our method of proof requires the assumption  $\{b_n\} \in U$  but presumably this can be weakened to  $\{b_n\} \in l_2$ . Finally, if  $\phi(z) \neq 0$  in  $R(z) \geq 0$  then  $\{f(nx)\}$  is complete.

<sup>1</sup> Bourgin, D. G., and Mendel, C., "Orthonormal Sets of Periodic Functions of the Type  $\{f(nx)\}$ ," *Trans. Am. Math. Soc.*, **57**, 332-363 (1945).

<sup>2</sup> Watson, G. N., "General Transforms," *Proc. Lond. Math. Soc.*, **35**, 156-199 (1933).

<sup>3</sup> Paley, R. E. A. C., and Wiener, N., "Fourier Transforms in the Complex Domain," *Am. Math. Soc. Coll. Pub.*, p. 100.

## INTRINSIC AREA

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Communicated November 20, 1945

Many definitions of angle and area have been proposed for Finsler spaces. So far there is no criterion, according to which one definition of angle is more natural than the others. But there is such a criterion for area, as the present note will show. The corresponding definition of area is also new for Riemann or even Euclidean spaces.

In spite of the many available other definitions it seems to deserve attention in the latter case, in particular, for surfaces in parametric form; because it does not use special properties of the Euclidean space or the spaces of constant curvature (like the existence of polyhedra or projections on planes); and because it *depends only on the geometry on the surface*. In differential geometry this last property has always been considered an indispensable attribute of area.

The concept of a parametrized set  $S$  requires two spaces: a parameter space  $P$  and a space  $R$  in which  $S$  lies. To describe their properties denoted generally by  $M^*$  the (up to isometries) unique smallest complete metric space that contains the metric space  $M$ . It will then be required

( $\alpha$ )  $P$  is separable and metric, and  $P^*$  is arcwise connected. Points of  $P^*$  are denoted by  $p, q, r$ , and the distance of  $p, q$  by  $pq$ .

( $\beta$ )  $R$  is metric.  $x, y, z$  are used for points of  $R$ ;  $xy$  is the distance of  $x$  and  $y$ .

( $\gamma$ ) There is a uniformly continuous mapping  $x = x(p)$  of  $P$  in  $R$  and  $S = x(P)$ .

The non-parametric case is the special case where the sets  $P$  and  $S$  coincide and  $x(p) = p$ .

The mapping  $x(p)$  can be extended to a uniformly continuous mapping of  $P^*$  in  $S^*$ . For two given points  $p, q$  of  $P^*$  let  $\Lambda$  be the set of all continuous curves  $p(\tau)$  from  $q$  to  $r$  in  $P^*$  and put

$$\kappa(q, r, x(P)) = \inf_{p(\tau) \in \Lambda} \lambda[x(p(\tau))]$$

where  $\lambda[x(p(\tau))]$  is the length of the image  $x(p(\tau))$  of  $p(\tau)$ . The function  $\kappa$  satisfies the triangle inequality, but may be 0 or  $\infty$ . By identifying points  $q, r$  with  $\kappa(q, r, x(P)) = 0$  new spaces  $\bar{P}$  and  $\bar{P}^*$  are obtained from  $P$  and  $P^*$  with the agreement that convergence in terms of  $\kappa$  means convergence in terms of  $\kappa/(1 + \kappa)$  with  $\infty/(1 + \infty) = 1$ . Now the  $n$ -dimensional area of  $S$  is defined as

$$\alpha_n(x(P)) = \alpha_n(S) = h_n(\bar{P}, \bar{P})$$

where  $h_n(M, E)$  denotes the  $n$ -dimensional Hausdorff measure of the set  $M$  in  $E$ . (That is, all coverings  $C$  of  $M$  by an at most countable number of spheres  $S(p_i, \rho_i)$ , with  $p_i \in E$ ,  $\rho_i < \epsilon$  are formed. Then

$$h_n^*(M, E) = \left\{ \pi^{n/2} / \Gamma(n/2 + 1) \right\} \inf_C \sum \rho_i^n,$$

and  $h_n^*(M, E) = \infty$ , if no  $C$  exists. Finally

$$h_n(M, E) = \lim_{\epsilon \rightarrow 0} h_n^*(M, E).$$

If  $\kappa(q, r, x(P)) = \infty$  for some pair  $q, r$ , then it does not follow that  $\alpha_n(S) = \infty$  for all  $n$ .

When  $\dim P = m < \infty$ , then  $\alpha_m(S)$  is simply called the area  $\alpha(S)$  of  $S$ . This agrees with the usual language in which the dimension of the parameter space decides whether we talk of a curve, a surface, ....

To deserve the name of an area  $\alpha(S)$  must satisfy certain generally accepted requirements.

1.  $\alpha_n(x(P))$  is independent of the parametrization. For obviously: If  $q \rightarrow p(q)$  is a uniformly topological mapping of the metric space  $Q$  on  $P$  and  $y(q) = x(p(q))$  then  $\alpha_n(y(Q)) = \alpha_n(x(P))$ .

2.  $\alpha_1(S) = \alpha_1(S)$  coincides with the arc length if  $S$  is a curve, or  $P = P^*$  is an interval  $\alpha \leq \tau \leq \beta$  of the real axis.

3. If  $\alpha_n(S) > 0$ , then  $\alpha_i(S) = \infty$  for  $i < n$ ; if  $\alpha_n(S) < \infty$  then  $\alpha_i(S) = 0$  for  $i > n$ .<sup>3</sup>

4. For an  $n$ -dimensional polyhedron  $\alpha_n(S) = \alpha(S)$  coincides with the elementary area. It is very easy to prove this in the following general form:

$P$  is an arcwise connected locally finite  $n$ -dimensional euclidean polyhedron in some  $E^m$  consisting of at most countably many closed  $n$ -simplices  $Q_1, Q_2, \dots$  where different  $Q_i$  have at most boundary points in common.  $x(p)$  is a continuous mapping of  $P$  in a metric space  $R$ , which is topological considered as mapping of a fixed  $Q_i$ , and  $x(Q_i)$  is congruent to a euclidean  $n$ -simplex  $S_i$ . If  $\mu(S_i)$  denotes the elementary volume of  $S_i$ , then  $\alpha(x(P)) = \sum \mu(S_i)$ .

5.  $\alpha_n(S)$  satisfies Kolmogoroff's Principle<sup>4</sup> in this form:

If  $y(p)$  is another uniformly continuous mapping of  $P$  in a metric space  $R'$  and  $y(q)y(r) \leq \beta x(q)x(r)$  then

$$\alpha_n(y(P)) \leq \beta^n \alpha_n(x(P)).$$

The following property distinguishes  $\alpha(P)$  from other areas, for which it is either hitherto unproved or false.

6.  $\alpha_n(S)$  depends only on the intrinsic geometry on the surface  $x(P)$ . This becomes clearer in the non-parametric case. Then  $\kappa(x, y)$  is simply the greatest lower bound of all curves from  $x$  to  $y$  on  $S$  or the geodesic distance of  $x$  and  $y$  on  $S$ .

In Riemann spaces of class  $C'$  the number  $\alpha(S)$  coincides for surfaces of class  $C'$  with the usual integral, moreover  $\alpha(S)$  is for surfaces of class  $C'$  the only area which satisfies the following three conditions:

(I)  $\alpha(S)$  is intrinsic, i.e., depends only on the geodesic distances  $\kappa(q, r, x(P))$ .

(II)  $\alpha(S)$  satisfies Kolmogoroff's Principle (5) for  $\beta = 1$ .

(III) For piecewise Euclidean spaces (finite polyhedra)  $\alpha(S)$  coincides with the elementary area.

In Finsler spaces no generally accepted analytical expression for the area exists. But similarly as in Riemann spaces it is true that  $\alpha(S)$  is for surfaces of class  $C'$  in Finsler spaces of class  $C'$  the only area which is intrinsic (I), satisfies Kolmogoroff's Principle for  $\beta = 1$  (II) and is such that:

(III') For piecewise Minkowskian spaces  $\alpha(S)$  coincides with the elementary area. (There is only one natural elementary area.)

For surfaces of class  $C'$  in a three-dimensional Finsler space<sup>1</sup> (section 2) indicates a definition of area. Provided the author interprets that brief remark correctly, this area can be shown to coincide with  $\alpha_2$ .

If  $x(P)$  is rectifiable, or in the present terminology, if  $P$  is the unit cube



of  $E^k$ ,  $R$  is the  $E^n$  and the mapping  $x(p)$  satisfies a Lipschitz condition  $x(p)x(q) < \rho \cdot pq$ , then  $\alpha(S) = \alpha_x(S)$  coincides with the classical integral and, therefore,<sup>2,5</sup> with the various other areas defined by Peano Lebesgue, Radò and Federer. For non-rectifiable surfaces the relations are mostly unclear.

Proofs will be forthcoming in a comprehensive paper with the same title.

<sup>1</sup> Bouligand, Georges, and Choquet, Gustave, "Problèmes liés à des métriques variationnelles," *C. R. Acad. Sci. Paris*, 218, 696-698 (1944).

<sup>2</sup> Federer, Herbert, "Surface Area I," *Trans. Amer. Math. Soc.*, 55, 420-437 (1944).

<sup>3</sup> Hurewicz, Witold, and Wallman, Henry, *Dimension Theory*, Princeton, 1941.

<sup>4</sup> Kolmogoroff, A., "Beiträge zur Masstheorie," *Math. Ann.*, 107, 351-366 (1932).

<sup>5</sup> Nöbeling, G., "Ueber den Flächeninhalt dehnungsbeschränkter Flächen," *Math. Zeitschrift*, 48, 747-771 (1943).

## THE MEAN CONVERGENCE OF ORTHOGONAL SERIES OF POLYNOMIALS

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1. The literature concerning series of orthogonal polynomials is devoted chiefly to the problems of ordinary convergence and summability. The question of mean (or strong) convergence has apparently not been investigated. The purpose of the present note is to announce some results concerning this problem.

Stated in its most general form the problem of mean convergence is this. Let  $F(x)$  be an increasing function on  $(a, b)$ , with infinitely many points of increase; and let  $\{p_n(x)\}$  be the associated orthonormal polynomials.<sup>1</sup> Then for  $m, n = 0, 1, 2, \dots$

$$\int_a^b p_m(x)p_n(x)dF(x) = \delta_{mn}. \quad (1)$$

To every suitable function  $f(x)$ , there corresponds a formal expansion

$$\begin{aligned} f(x) &\sim \sum a_n p_n(x), \\ a_n &= \int_a^b f(x)p_n(x)dF(x). \end{aligned} \quad (2)$$

Suppose now that  $f(x)$  belongs to  $L_p^F$ , i.e., is  $F$ -measurable and  $\int_a^b |f|^p dF(x)$  exists as a Lebesgue-Stieltjes integral. For what values of  $p$  (other than 2, when the result is always true) does the series (2) converge strongly to  $f(x)$  in the sense

$$\lim_{N \rightarrow \infty} \int_a^b |f(x) - \sum_0^N a_n p_n(x)|^p dF(x) = 0.$$

In the language of functional analysis: for what values of  $p$  do the orthonormal polynomials associated with  $F(x)$  form a basis<sup>2</sup> for  $L^p$ ?

**THEOREM 1.** *If  $F'(x) = (1 - x^2)^{\lambda-1/2}$ ,  $-1 < x < 1$ ,  $\lambda \geq 0$  then the associated (ultraspherical) polynomials form a basis for  $L^p(-1, 1)$  if*

$$2 - \frac{1}{\lambda + 1} < p < 2 + \frac{1}{\lambda}, \quad (3)$$

*but not if  $1 \leq p < 2 - \frac{1}{\lambda + 1}$  or  $p > 2 + \frac{1}{\lambda}$ .*

*Remarks on Theorem 1.*—(i) If  $\lambda = 0$  the result follows from M. Riesz' theorem on the mean convergence of Fourier series.<sup>3</sup> (ii) If  $\lambda = 1/2$  Theorem 1 establishes a conjecture (unpublished) of Zygmund that the Legendre polynomials form a basis for  $L^p(-1, 1)$  if  $4/3 < p < 4$ . (iii) The limiting case  $\lambda = \infty$  is interesting. If  $x$  in  $(1 - x^2)^{\lambda-1/2}$  is replaced by  $x\lambda^{-1/2}$ , and then the limit taken as  $\lambda \rightarrow \infty$ , then the corresponding polynomials become those of Hermite on  $(-\infty, \infty)$ . The formula (3) suggests that for these polynomials mean-convergence holds only for  $p = 2$ . This, and a similar result for Laguerre polynomials, can be confirmed by suitable counter-examples. (iv) The end values  $p = 2 - \frac{1}{\lambda + 1}$ ,  $2 + \frac{1}{\lambda}$  are left open.

2. If  $F(x)$  is absolutely continuous there is another interpretation of mean convergence. Let  $F'(x) = w(x)$ . Then the functions  $\{w^{1/2}(x)p_n(x)\}$  form an orthonormal set in the ordinary sense; this follows from (1). With each function in  $L^p(a, b)$  one can associate a series

$$f(x) \sim \sum b_n w^{1/2}(x)p_n(x),$$

$$b_n = \int_a^b f(x) w^{1/2}(x)p_n(x) dx.$$

The question now is to determine when

$$\lim_{N \rightarrow \infty} \int_a^b |f(x) - \sum_0^N b_n w^{1/2}(x)p_n(x)|^p dx = 0.$$

In a sense this question is less "natural" than that of §1 for it is only by virtue of the accident that  $F(x)$  is absolutely continuous that it has meaning. The following is true.

**THEOREM 2.** *If  $w(x) = (1 - x)^\alpha(1 + x)^\beta$ ,  $-1 < x < 1$ ,  $\alpha \geq 0$ ,  $\beta \geq 0$ , then the functions  $\{w^{1/2}(x)p_n(x)\}$  form a basis for  $L^p(-1, 1)$  if  $4/3 < p < 4$ , but not if  $1 \leq p < 4/3$ , or  $p > 4$ .*

*Remarks on Theorem 2.*—(i) The corresponding polynomials are, of course, those of Jacobi.<sup>4</sup> (ii) It is of interest to observe that the range of  $p$  is independent of the specific choice of  $\alpha, \beta$ . (iii) The first part of the theorem is true even if  $\alpha \geq -1/2$ ,  $\beta \geq -1/2$ ; the second part is open in this more general case. (iv) The end values  $p = 4/3, 4$  are open.



3. These two problems make somewhat heavier demands than the classical convergence questions on the methods of functional analysis. By the Banach-Steinhaus theorem<sup>6</sup> the problems can be reduced to showing that the norms (in the appropriate space) of the partial sums are bounded, i.e., if  $s_n(f)$  denotes the partial sums of the series corresponding to  $f(x)$ , then  $\|s_n(f)\| \leq M\|f\|$  for some positive  $M$  and all  $f$  in the space. This is accomplished by two devices, of which the first alone suffices for trigonometrical series: M. Riesz' theory of conjugate functions,<sup>3</sup> and the following inequality.

LEMMA. If  $-1 < c < 1$ ,  $c < \frac{1}{p} < c + 1$ ,  $p > 1$ , and  $f(x)$  belongs to  $L^p(-1, 1)$ , then so does

$$g(x) = \int_{-1}^1 \left| \frac{\left(\frac{1-y^2}{1-x^2}\right)^c - 1}{x-y} \right| f(y) dy.$$

Moreover  $\|g\|_p \leq A\|f\|_p$ , where  $A$  is independent of  $f$ .

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<sup>1</sup> See, for example p. 24 of G. Szegő's *Orthogonal Polynomials*, New York, 1939.

<sup>2</sup> For this use of the word "basis" see S. Banach, *Théorie des opérations linéaires*, Warsaw, 1932.

<sup>3</sup> *Math. Zeit.*, 27, 218 (1927).

<sup>4</sup> Szegő, op. cit., Chapter IV.

<sup>5</sup> Banach, op. cit., p. 79.

## THE FUNDAMENTAL THEOREM ON QUADRATIC FIRST INTEGRALS

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In the following we have shown that the set of all homogeneous quadratic first integrals of the differential equations of the paths of an affinely connected space admits a finite basis. The demonstration is so devised that the number of integrals in the basis is identical with the number of solutions in a fundamental system of solutions of a certain set of linear homogeneous equations; hence the determination of this number is reduced to the solution of an algebraic problem.

A corresponding basis theorem holds for the integrals of energy type of a conservative dynamical system. Due to the importance of such integrals for the dynamical problem the proof in question has been indicated and the result stated in the form of a theorem.

It suffices for the demonstration to assume the analyticity of the various functions involved. But this assumption is far too drastic. The differentiability requirements should be such as to permit the construction of the above-mentioned system of linear homogeneous equations by which the number of integrals in the basis is determined. In general, however, the process by which this system is obtained will speedily terminate yielding moderate requirements of differentiability.

*Integrals in General Affine Space.*—Consider the conditions under which the differential equations

$$\frac{d^2x^\alpha}{ds^2} + \Gamma_{\mu\nu}^\alpha \frac{dx^\mu}{ds} \frac{dx^\nu}{ds} = 0 \quad (1)$$

defining the paths of a general affinely connected space of symmetric affine connection  $\Gamma$ , admit a homogeneous quadratic integral

$$g_{ij} \frac{dx^i}{ds} \frac{dx^j}{ds} = \text{const.} \quad (2)$$

Among these various conditions we signalize the following as the basis of our discussion.<sup>1</sup> *First*, there exist relations of the form

$$g_{ij,p,q} = g_{ij,pq} - g_{aj}A_{ipq}^\alpha - g_{ia}A_{jpq}^\alpha \quad (3)$$

$$g_{ij,pq,r} = g_{\alpha\beta}E_{ijpq}^{\alpha\beta} + g_{\alpha\beta,\gamma}F_{ijpq}^{\alpha\beta\gamma} \quad (4)$$

where the  $g_{\alpha\beta,\gamma}$  and the  $g_{ij,pq}$  are the components of the first covariant derivative (first extension) and second extension, respectively, of the tensor defined by the coefficients in (2), etc.; also the quantities  $A_{ipq}^\alpha$  are the components of the first normal tensor. System (3) is an identity in the space. System (4) is satisfied in virtue of the fact that (2) is a quadratic integral. *Second*, there exists a sequence of sets of equations

$$S_0 = 0, S_1 = 0, S_2 = 0, \dots \quad (5)$$

each of which is linear and homogeneous in the quantities  $g_{ij}$ ,  $g_{ij,p}$  and  $g_{ij,pq}$  with coefficients which are constants or tensor invariants of the space. A necessary and sufficient condition for the existence of an integral (2) is that there is an integer  $N$  such that the first  $N + 1$  sets of equations of the sequence (5) are consistent as equations for the determination of the quantities  $g_{ij}$ ,  $g_{ij,p}$  and  $g_{ij,pq}$  and that all their solutions satisfy the  $(N + 2)$ nd set of equations of the sequence. *Third*, if  $g_{ij}^\alpha$ ,  $g_{ijp}^\alpha$  and  $g_{ijpq}^\alpha$  where  $\alpha = 1, \dots, s$  and  $s \geq 1$  is a fundamental system of solutions of the first  $N + 1$  sets of the sequence, the general solution of these equations is given by

$$g_{ij} = \phi^\alpha g_{ij}^\alpha, g_{ijp} = \phi^\alpha g_{ijp}^\alpha, g_{ijpq} = \phi^\alpha g_{ijpq}^\alpha \quad (6)$$

where  $\alpha$  is summed over the values  $1, \dots, s$  and the  $\phi$ 's are arbitrary func-

tions of the  $x$ 's. A sufficient condition for (2) to be an integral is now that  $g_{ij} = \phi^\alpha g_{ij}^\alpha$  where the  $\phi$ 's satisfy a certain completely integrable system of the form

$$\frac{\partial \phi^\beta}{\partial x^p} + \phi^\alpha \lambda_p^{\alpha\beta} = 0. \quad (7)$$

Assuming the above conditions for the existence of the integral (2) to be satisfied we can now determine  $s$  sets of functions  $\phi^\alpha$  as solutions of (7) by the following  $s$  sets of initial conditions

$$(1, 0, 0, \dots, 0), (0, 1, 0, \dots, 0), \dots, (0, 0, 0, \dots, 1).$$

We thus obtain  $s$  integrals (2) with  $g$ 's given by  $g_{ij}^\alpha$  where  $\alpha = 1, \dots, s$ . Initially we have

$$g_{ij}^\alpha = g_{ij}^\alpha, \quad g_{ij,p}^\alpha = g_{ij,p}^\alpha, \quad g_{ij,pq}^\alpha = g_{ij,pq}^\alpha$$

where the quantities on the left are the components of the tensors  $g^\alpha$  and their first and second extensions while the quantities on the right are the above fundamental system of algebraic solutions. Hence these left-hand quantities can be taken as a fundamental system of solutions of the first  $N + 1$  sets of equations (5). We state this result as the following theorem.

**THEOREM I.** *If the first  $N + 1$  sets of equations (5) admit a fundamental system of  $s (\geq 1)$  algebraic solutions each of which satisfies the  $N + 2$ nd set of these equations, then there exists  $s$  quadratic integrals*

$$g_{ij}^\alpha \frac{dx^i}{ds} \frac{dx^j}{ds} = \text{const.}, \quad (\alpha = 1, \dots, s), \quad (8)$$

such that the tensors  $g^\alpha$ , which are determined by the coefficients of these integrals, yield a fundamental system of solutions  $g_{ij}^\alpha, g_{ij,p}^\alpha, g_{ij,pq}^\alpha$  of the first  $N + 1$  sets of the equations. We use this fundamental system of solutions in the following discussion.

The  $s$  integrals (8) are linearly independent in the sense that the equations  $c_\alpha g_{ij}^\alpha = 0$ , in which the  $c$ 's are constants, imply that  $c_\alpha = 0$ . For, it follows from these equations that  $c_\alpha g_{ij,p}^\alpha = 0$  and  $c_\alpha g_{ij,pq}^\alpha = 0$ ; hence all  $c_\alpha = 0$  since otherwise we would have a linear relation between the solutions of our fundamental system.

Now let (2) be any quadratic integral. Then

$$g_{ij} = \phi^\alpha g_{ij}^\alpha, \quad g_{ij,p} = \phi^\alpha g_{ij,p}^\alpha, \quad g_{ij,pq} = \phi^\alpha g_{ij,pq}^\alpha \quad (9)$$

for suitable  $\phi$ 's. Hence from (3), (4) and (9) we have

$$\begin{aligned} (\phi^\alpha g_{ij}^\alpha)_{,p} &= \phi^\alpha g_{ij,p}^\alpha \\ (\phi^\alpha g_{ij,p}^\alpha)_{,q} &= \phi^\alpha g_{ij,pq}^\alpha - \phi^\alpha g_{ij,p}^\alpha A_{iq}^\alpha - \phi^\alpha g_{ij,q}^\alpha A_{ip}^\alpha \\ (\phi^\alpha g_{ij,pq}^\alpha)_{,r} &= \phi^\alpha g_{ij,pqr}^\alpha + \phi^\alpha g_{ij,pq}^\alpha A_{ir}^\alpha + \phi^\alpha g_{ij,p}^\alpha A_{iqr}^\alpha + \phi^\alpha g_{ij,q}^\alpha A_{ipr}^\alpha. \end{aligned}$$

Performing the indicated differentiations in the left members of these equations, and using the fact that the quantities  $g_{ij}^\alpha$  (for each value of  $\alpha$ ) satisfy (3) and (4), it follows that

$$\phi_{,p}^\alpha g_{ij}^\alpha = 0, \quad \phi_{,q}^\alpha g_{ij,p}^\alpha = 0, \quad \phi_{,r}^\alpha g_{ij,pq}^\alpha = 0. \quad (10)$$

But (10) implies that  $\phi_{,p}^\alpha = 0$  since otherwise there would exist a linear relation between the solutions of the fundamental system. Hence  $\phi^\alpha = \text{const.}$  Hence,

**THEOREM II.** *If quadratic integrals (2) of the differential equations (1) exist, then the  $s$  integrals (8) constitute a basis for the set of all such integrals, i.e., if (2) is any quadratic integral we will have  $g_{ij} = c_\alpha g_{ij}^\alpha$  where the  $c$ 's are constants.*

*Remark 1.*—Similar theorems can be proved for linear first integrals of (1) on the basis of results (loc. cit., p. 591) analogous to those used in the proof of the above theorems for quadratic integrals. The methods are general and one can conclude that corresponding theorems are true for homogeneous first integrals of any degree.

*Remark 2.*—The above theorems on quadratic integrals apply in particular when (1) are the differential equations of the geodesics of a Riemann space. In this case the  $\Gamma$ 's are the well-known Christoffel symbols and the basis of quadratic integrals can be considered to contain the quadratic integral determined by the fundamental metric tensor of the space.

*Remark 3.*—For the particular class of integrals

$$a_{\alpha\beta\dots\gamma} \frac{dx^\alpha}{ds} \frac{dx^\beta}{ds} \dots \frac{dx^\gamma}{ds} = \text{const.} \quad (11)$$

such that  $a_{\alpha\beta\dots\gamma,\delta} = 0$  the sequence corresponding to (5) is readily constructed (loc. cit., p. 585) and yields results of the type expressed by the above Theorems I and II. The quadratic integrals of this class are of especial interest from the geometrical standpoint.

*Quadratic First Integrals in Flat Space.*—If the space is flat the equations  $S_k = 0$  for  $k = 1, 2, \dots$  are satisfied identically and the set of equations  $S_0 = 0$  becomes

$$g_{ij,p} + g_{jp,i} + g_{pi,j} = 0, \quad g_{ij,pq} + g_{jp,iq} + g_{pi,jq} = 0, \\ g_{ij} = g_{ji}, \quad g_{ij,p} = g_{ji,p}, \quad g_{ij,pq} = g_{ji,pq} = g_{ij,qp}.$$

Hence we can take  $N = 1$  in Theorem I and the number  $s$  of quadratic integrals (8) in the basis is equal to the number of algebraically independent quantities  $g_{ij}$ ,  $g_{ij,p}$  and  $g_{ij,pq}$  in the above equations. We thus find

$$s = \frac{n(n+1)^2(n+2)}{12}. \quad (12)$$

Similar remarks apply to integrals of the type (11); we note in particular

that  $n(n+1)/2$  is the number of linearly independent quadratic integrals of this type.

It is evident that the number  $s$  given by (12) is an upper bound to the number of quadratic integrals in the basis for any system (1). Similarly,  $n(n+1)/2$  is an upper bound for the number of quadratic integrals of type (11), i.e., which satisfy the condition  $a_{\alpha\beta,\gamma} = 0$ . The above discussion shows that in the case of a flat space these upper bounds are actually attained.

We leave open the question of whether the existence of this maximum number of integrals is a sufficient condition for the space to be flat.

*Quadratic Integrals of a Dynamical System.*—The differential equations of the trajectories of a conservative dynamical system can be written

$$\frac{d^2x^\alpha}{dt^2} + \Gamma_{\mu\nu}^\alpha \frac{dx^\mu}{dt} \frac{dx^\nu}{dt} = -g^{\alpha\sigma} V_{,\sigma} \quad (13)$$

where  $V(x^1, \dots, x^n)$  is the potential and the  $\Gamma$ 's are Christoffel symbols based on the coefficients  $g_{\mu\nu}$  of the quadratic form

$$T = \frac{1}{2} g_{\mu\nu}(x) \frac{dx^\mu}{dt} \frac{dx^\nu}{dt} \quad (14)$$

defining the kinetic energy  $T$ . We assume that the  $g_{\mu\nu}$  do not involve the time  $t$  explicitly. The system (13) then admits the quadratic integral

$$\frac{1}{2} g_{\mu\nu} \frac{dx^\mu}{dt} \frac{dx^\nu}{dt} + V = \text{const.} \quad (15)$$

which expresses the condition that the sum of kinetic and potential energies is constant along any trajectory.

It should be possible to prove theorems analogous to Theorems I and II for integrals of the type (15). Here, however, we prove only the finite basis theorem for such integrals as a consequence of Theorem II. Thus consider any integral of (13) of the type (15), namely,

$$\frac{1}{2} h_{\mu\nu} \frac{dx^\mu}{dt} \frac{dx^\nu}{dt} + W = \text{const.} \quad (16)$$

The differential conditions on the quantities  $h_{\mu\nu}$  and  $W$  for (16) to be an integral of (13) are readily deduced and indicate that

$$h_{\mu\nu} \frac{dx^\mu}{dt} \frac{dx^\nu}{dt} = \text{const.} \quad (17)$$

is an integral of the differential equations of the geodesics, i.e., the equations obtained from (13) by replacing the right members by zero. Hence, dealing only with those quadratic integrals (17) of the equations of geodesics which are associated with integrals (16) of (13) it now follows from the

above basis theorem for homogeneous quadratic first integrals that there exists a finite number of these integrals (17) in terms of which any other such integral can be expressed linearly with constant coefficients. In other words there exist  $s(\geq 1)$  integrals

$$\frac{1}{2} g_{\mu\nu}^{\alpha} \frac{dx^{\mu}}{dt} \frac{dx^{\nu}}{dt} + V^{\alpha} = \text{const.}, (\alpha = 1, \dots, s), \quad (18)$$

of (13) such that if (16) is any integral of (13) we must have

$$h_{\mu\nu} = c_{\alpha} g_{\mu\nu}^{\alpha}, \quad (19)$$

where the  $c$ 's are constants. Now

$$\frac{1}{2} c_{\alpha} g_{\mu\nu}^{\alpha} \frac{dx^{\mu}}{dt} \frac{dx^{\nu}}{dt} + c_{\alpha} V^{\alpha} = \text{const.} \quad (20)$$

is an integral of (13) for any selection of the constants  $c_{\alpha}$ . But if we choose the  $c$ 's to satisfy (19) it follows from (16) and (20) that  $W - c_{\alpha} V^{\alpha} = \text{const.}$  along any trajectory. Differentiating this equation with respect to  $t$  we have  $(W - c_{\alpha} V^{\alpha})_{,\beta} dx^{\beta}/dt = 0$  along the trajectory and since the  $dx^{\beta}/dt$  can be chosen arbitrarily it follows that  $(W - c_{\alpha} V^{\alpha})_{,\beta} = 0$ . Hence

$$W = c_{\alpha} V^{\alpha} + k, \quad (21)$$

where  $k$  is an absolute constant.

We can suppose that there exists no linear relation with constant coefficients, not all of which are zero, between the left members of (18) since otherwise the number  $s$  of these equations could be reduced. The set of integrals (18) is then said to be linearly independent. We state the above result as the following theorem.

**THEOREM III.** *There exists a finite number  $s(\geq 1)$  of linearly independent integrals (18) of the conservative dynamical system (13) such that any other integral (16) is determined by the relations (19) and (21) in which the  $c$ 's and  $k$  are constants.*

The set of linearly independent integrals (18) occurring in the above theorem will be said to form a basis of integrals for the dynamical system.

<sup>1</sup> Veblen, O., and Thomas, T. Y., "The Geometry of Paths," *Trans. Am. Math. Soc.*, 25, 551-608 (1923).



## CONVERSE THEORY OF GNOMIC AND EQUIAREAL PERSPECTIVITIES\*

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1. *Perspective Conformalities.*—We shall present some theorems on the perspective mapping of a surface upon a plane from a given point. In an earlier paper,<sup>1</sup> we have proved the following result.

*The only perspective conformalities upon a plane are Ptolemy's stereographic projection of a sphere (and the obvious limiting case of a parallel plane).*

Thus there are no surfaces except for spheres and planes, for which there exists a perspectivity upon a plane from a given point, which is conformal.

In our present work, we shall consider some properties of gnomonic projection of a sphere upon a plane (geodesic mapping). Also, we shall study surfaces for which area-preserving perspectivities exist.

2. *Perspective Representation of Geodesics.*—Among Kasner's theorems on the problem of partial geodesic representation and the near-collineation problem, may be found the following proposition.<sup>2</sup>

*If there exists a point-to-point representation of a surface upon a plane such that more than  $3\infty^1$  geodesics correspond to straight lines, then all geodesics correspond to straight lines. Therefore, by a theorem of Beltrami the surface is of constant curvature.*

We shall restrict our point transformations to perspectivities. The condition of constant curvature for our case is only necessary but not sufficient. We shall discuss the following theorem.

*Characterization of gnomonic projection. If more than  $3\infty^1$  geodesics are projected into straight lines under a perspectivity, then all geodesics project into straight lines, and the surface is a sphere (or the obvious case of any plane, parallel or not); furthermore the point of perspectivity is at the center of the sphere.*

We shall deduce from our work the following classification of surfaces according to the number of geodesics which are projected into straight lines by a perspectivity. *There are four distinct classes.*

(I) The non-ruled surface. At most  $\infty^1$ .

(II) The ruled surfaces excluding the quadrics. There are always  $\infty^1$  (the rulings) and at most  $2\infty^1$ .

(III) The quadrics excluding the gnomonic projections of spheres. There are always  $2\infty^1$  (the two systems of rulings) and at most  $3\infty^1$ .

(IV) The gnomonic projection of a sphere, and the limiting case of any plane. All  $\infty^2$ .

3. *Surfaces with Plane Geodesics.*—Elsewhere<sup>3</sup> we have proved the following result which is closely related to the above.

*The spheres are the only surfaces which possess more than  $2\infty^1$  non-rectilinear plane geodesics.*

The discussion of surfaces with reference to the maximum number of plane geodesics, straight or not, leads to the same classification as above. The maximum number of plane geodesics for each class is as follows.

- (I) At most  $2\infty^1$ .
- (II) Always  $\infty^1$  and at most  $3\infty^1$ .
- (III) Always  $2\infty^1$  and at most  $4\infty^1$ .
- (IV) All  $\infty^2$ .

4. *Discussion of Our Characterization of Gnomonic Projection.*—Let  $(x, y, z)$  denote cartesian coördinates of a point. Let  $z = f(x, y)$  be the equation of our surface  $\Sigma$ . Introduce the usual notation:  $p = \partial z / \partial x$ ,  $q = \partial z / \partial y$ ;  $r = \partial^2 z / \partial x^2$ ,  $s = \partial^2 z / \partial x \partial y$ ,  $t = \partial^2 z / \partial y^2$ .

Take the origin as the center of perspectivity and  $z = c \neq 0$  as the given plane  $\pi$ . The perspectivity from the given point  $O(0, 0, 0)$  upon the plane  $\pi: (X, Y, c)$  of the surface  $\Sigma: (x, y, z = f(x, y))$ , is

$$X = cx/z, \quad Y = cy/z. \quad (1)$$

The jacobian  $j$  of this perspectivity is

$$j = \frac{c^2}{z^3} (z - xp - yq) \neq 0. \quad (2)$$

Observe that the surface  $\Sigma$  cannot be a plane through the origin, or a cone with vertex at the origin.

It is found by (1) that the differential equation defining the straight lines of the plane  $\pi$ , is

$$(z - xp - yq)y'' = (y - xy')(r + 2sy' + ty'^2). \quad (3)$$

The geodesics of the surface  $\Sigma$  are given by the differential equation

$$(1 + p^2 + q^2)y'' = (-q + p \cdot y')(r + 2sy' + ty'^2). \quad (4)$$

Eliminating  $y''$  from (3) and (4), we find that the possible geodesics which are projected into straight lines satisfy either

$$r + 2sy' + ty'^2 = 0, \quad (5)$$

or

$$[(1 + q^2)(zp + x) - pq(zq + y)]y' = [-pq(zp + x) + (1 + p^2)(zq + y)]. \quad (6)$$



For any surface where all the geodesics are projected into straight lines, either (5) or (6) or both, are identities in  $y'$ . These identities will demonstrate that any such surface  $\Sigma$  is either a plane in general position, or a sphere with center at the origin. This result can be deduced also from the theorem stated in Section 3.

The classification of surfaces with reference to the number of geodesics which are projected into straight lines, may be deduced from the following observations. Firstly, if (5) represents an infinitude of geodesics, these must be straight lines and hence the surface is ruled. Secondly, the quadrics are the only ruled surfaces with two distinct systems of rulings. Thirdly and finally, the non-rectilinear geodesics which project into straight lines must satisfy (6).

5. *Surfaces for Which Area-Preserving Perspectivities Exist.*—By (2), the area formula in the plane  $\pi$ , is

$$\text{Area in } \pi = c^2 \iint \frac{1}{z^3} (z - xp - yq) dx dy. \quad (7)$$

The area formula in the surface  $\Sigma$  is

$$\text{Area in } \Sigma = \iint (1 + p^2 + q^2)^{1/2} dx dy. \quad (8)$$

Therefore, all surfaces for which area-preserving perspectivities exist, must satisfy the partial differential equation of first order

$$c^4(z - xp - yq)^2 - z^6(1 + p^2 + q^2) = 0. \quad (9)$$

All plane solutions of this equation are  $z = 0$  (this is the singular solution),  $z = \pm c$ , and  $z = \pm ic$ . Thus the only real non-trivial plane surface for which an area-preserving perspectivity exists, is the plane  $\pi': z = -c$ .

It can be proved that *there are no spherical surfaces which satisfy the partial differential equation (9); so equiareal perspectivity of the sphere is impossible.*

In order to study this partial differential equation of first order (9), in the real domain, it is found convenient to introduce the following algebraic surface  $R$  of revolution of the sixth degree. It is the locus of a point such that its distance from the point of perspectivity  $O(0, 0, 0)$  is equal to the ratio of the cube of its distance from the plane  $\pi_0: z = 0$ , to the square of the distance  $c$  of the plane  $\pi: z = c$ , from  $\pi_0$ . The equation of this algebraic surface  $R$  is

$$x^2 + y^2 + z^2 = z^6/c^4. \quad (10)$$

This surface  $R$  has an isolated singularity at the point of perspectivity  $O$ , the tangent directions being on the minimal cone with vertex at  $O$ . Otherwise the surface  $R$  is defined for  $z \geq c$  and  $z \leq -c$ .

Note that the partial differential equation (9) may be written in the form

$$(y + qz)^2 + (x + pz)^2 + (yp - xq)^2 - \left(x^2 + y^2 + z^2 - \frac{z^6}{c^4}\right) \times \\ (1 + p^2 + q^2) = 0. \quad (11)$$

For real solutions we must have  $x^2 + y^2 + z^2 \geq z^6/c^4$ .

*In the real domain, the partial differential equation (9) is defined only in the region bounded by the algebraic surface  $R$  of sixth degree, which contains the point of perspectivity  $O$ . At any point  $P$  of  $R$ , there is only one real planar direction whose normal is the radius vector of  $P$ .*

It is found that at each point  $(x, y, z)$ , the surface elements of (9), envelope the quadric cone

$$(x dx + y dy + z dz)^2 - (x^2 + y^2 + z^2 - z^6/c^4)(dx^2 + dy^2 + dz^2) = 0. \quad (12)$$

These quadric cones can degenerate only along our algebraic surface  $R$ .

*By Charpit's method, the complete integrals of the partial differential equation of first order (9), are cylinders with elements parallel and symmetrical to a line through the point of perspectivity, all of which are parallel to the plane  $\pi$ . The directorial curve is expressed parametrically in terms of a Tchebycheff integral of non-elementary character.*

The axis of the cylinders may be taken as the  $y$ -axis. The directorial curve is in the  $xz$ -plane and its differential equation can be written in the form

$$x = z \frac{dx}{dz} \pm \frac{z^3}{c^2} \left[ 1 + \left( \frac{dx}{dz} \right)^2 \right]^{1/2}. \quad (13)$$

Taking the inclination  $t$  of the tangent line of this curve to the  $z$ -axis as parameter, it is found that this curve is given by the parametric equations

$$x = z \tan t \pm \frac{z^3}{c^2} \sec t, \quad z^2 = \frac{2c^2}{3} \cos^{1/2} t [K \pm \int \sec^{1/2} t dt], \quad (14)$$

where  $K$  is a constant of integration. The integral which appears above may be shown to be reducible to a Tchebycheff integral. By a theorem of Liouville, this is found to be not expressible in terms of the elementary functions.

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<sup>1</sup> Kasner and De Cicco, "Converse of Ptolemy's Theorem on Stereographic Projection," these PROCEEDINGS, 31, 338-342 (1945).

<sup>2</sup> See the following articles by Kasner: "The Characterization of Collineations," *Bull. Am. Math. Soc.*, 9, 545-546 (1903); "The Problem of Partial Geodesic Representation," *Trans. Am. Math. Soc.*, 7, 200-206 (1906).

<sup>3</sup> Kasner and De Cicco, "A New Characteristic Property of Minimal Surfaces," *Bull. Am. Math. Soc.*, 51, 692-699 (1945).



# PROCEEDINGS OF THE NATIONAL ACADEMY OF SCIENCES

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## *INFLUENCE OF HYDROSTATIC PRESSURE ON THE DENATURATION OF STAPHYLOCOCCUS ANTITOXIN AT 65°C.*

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The quantitative effects of increased hydrostatic pressure on reaction rates provide a measure of the direction and magnitude of the volume change of the reactants,  $\Delta V^\ddagger$ , in going from the normal to the activated state.<sup>1, 2</sup> With large molecules, such as those of proteins, these volume changes may be relatively large. Kinetic analysis of the rate of destruction of the bacterial luminescent system at various pressures and at temperatures about 15° above the normal optimum of the luminescent oxidation has shown that a large increase in volume, presumably of the protein catalyst and amounting to approximately 71 ml. per gram molecule, takes place.<sup>3</sup> In the denaturation of human serum globulin and egg albumin solutions at 65°C. a very considerable volume increase of activation also takes place, as evidenced by the decrease in the rate of precipitation at pressures of 10,000 lb. per square inch in comparison with the rate at normal pressure.<sup>4</sup>

In addition to these changes in molecular volume during the processes of activation, large volume increases in going from the initial to the final state of reversible denaturation equilibria evidently occur in the bacterial luminescent system at above optimum temperatures; or at lower temperatures in the presence of alcohol, urethane, ether, chloroform and certain other substances which bring about protein denaturation.<sup>5, 6, 7</sup> Furthermore, it has been shown by dilatometric studies that the denaturation of several proteins is accompanied by an increase in volume.<sup>8</sup>

The specific rate of inactivation of tetanus antitoxin at 60 to 66°C. is not constant, but decreases during the course of the reaction, showing that the mechanism of the inactivation is more complex than a single first order rate process.<sup>10</sup> The denaturation of diphtheria antitoxin by urea shows a

similar deviation from first order kinetics, although the specific rate is independent of the initial concentration of antibody.<sup>11, 12</sup> In the present investigation we have measured the rate of decrease in antibody activity of an equine antitoxin for staphylococcus hemolysin at 65°C. under normal and increased hydrostatic pressure with the hope of obtaining a more complete picture of the mechanism of the inactivation.

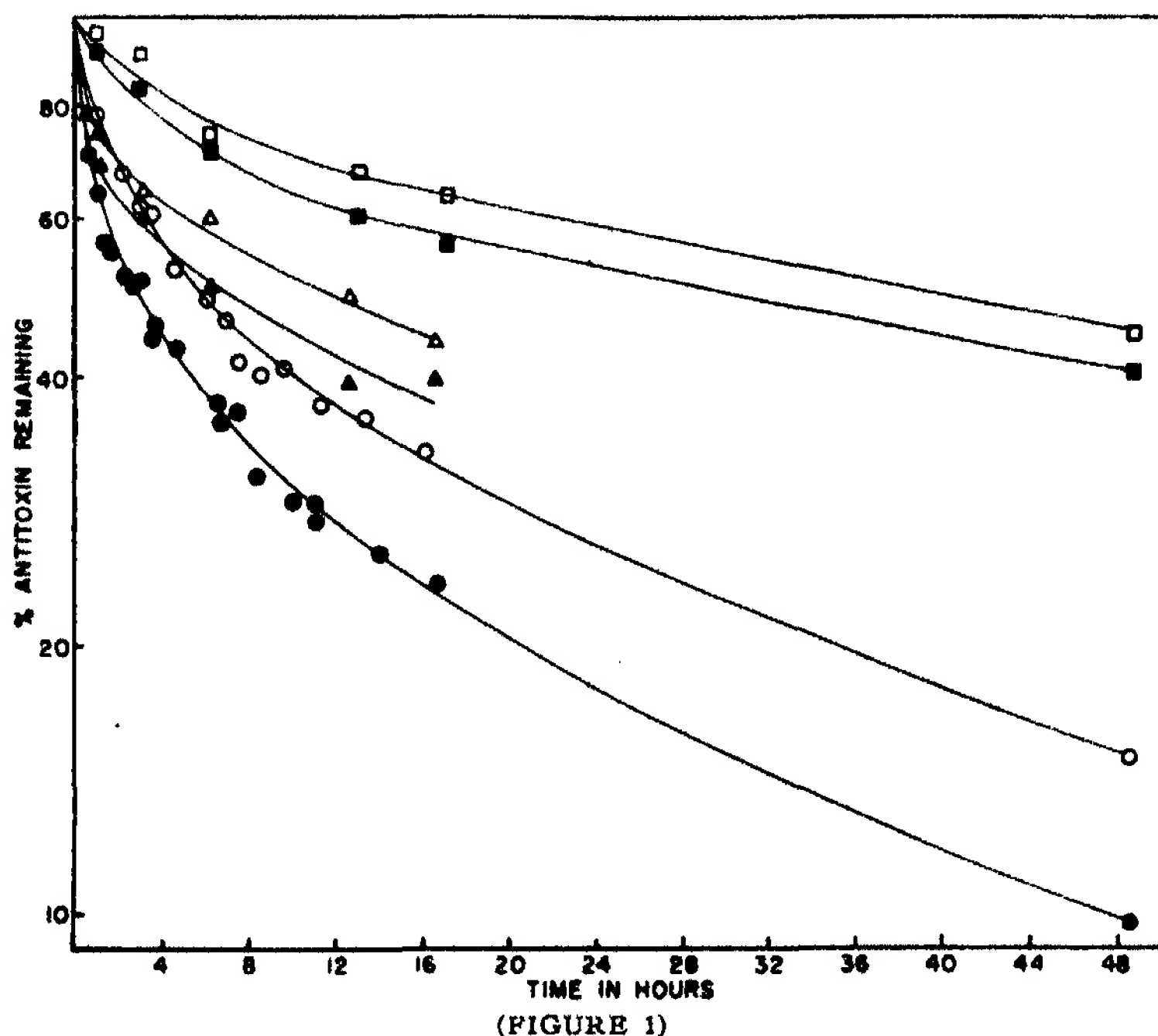
*Methods.*—The hemolysin solution was obtained from a semi-solid agar culture of *Staphylococcus aureus* in the usual manner.<sup>9</sup> The antitoxic globulin was obtained by fractionation of equine staphylococcus antitoxic plasma between 1.33 and 1.68 molar ammonium sulfate. The antiserum was supplied by The Lederle Laboratories, Pearl River, New York. The final stock globulin solution contained approximately 9 per cent globulin and 260 international units of antitoxin per milliliter.

Antibody solutions for denaturation were prepared by diluting the stock solution 1:62 in a buffer solution to give a protein concentration of 0.15 per cent. The pH 8.5 buffer was a boric acid, sodium borate and sodium chloride solution of ionic strength 0.1, the pH 4.82 buffer an acetic acid, sodium acetate and sodium chloride solution also of ionic strength 0.1, and the pH 6.65 buffer a *M*/10 sodium phosphate solution. Portions of each buffered solution of antibody were heated at normal pressure in a large, well-stirred water bath at  $65 \pm 0.015^\circ\text{C}$ . in Wassermann tubes. For denaturation under pressure the tubes were cut in length to hold about 3 ml., stoppered by means of rubber stoppers leaving no air space, and placed in a water-filled steel pressure chamber. The chamber was connected to a hydraulic pump, pressure was applied and the entire chamber was placed in the water bath. The time required for the samples to come essentially to bath temperature was found to be two and one-half minutes outside the pressure chamber and five and one-half inside; correction was made in the time of denaturation for this lag in heating. The pH of the solutions did not change appreciably during the course of the heating.

The antitoxic activity of each partially inactivated antibody sample was assayed by making a series of dilutions of the antibody in intervals of 6 per cent, after which a constant amount of the toxin, containing about 150 hemolytic units, was added to each tube. The tubes were mixed and allowed to stand for a few minutes, after which washed rabbit erythrocytes were added as indicator. Dilutions were made in saline, buffered with sodium phosphate to pH 7.4. Hemolysis of half of the cells after incubation was taken as the end-point; since this was fairly sharp, the end-point dilution could be estimated by visual observation to within 3 per cent or less. In each group of tests a series of dilutions of unheated buffered antibody solution was included to serve as reference standard, and the per cent activity of the partially inactivated samples was computed as the ratio of the end-point dilution of the sample to the end-point dilution of

the unheated material. Several of the assays were repeated and the results averaged; agreement was good in practically all cases.

**Results.**—In figure 1 the per cent antitoxic activity of samples heated at 65°C. under 10,000 lb. pressure is plotted for various times of heating up to 48 hours. The results are also given for control experiments carried out under the same conditions except that the samples were held at atmospheric pressure. The combined results of a number of experiments at each pH



The per cent antitoxic activity remaining as a function of the time of heating at 65°C. under various conditions of pH and hydrostatic pressure, as follows: squares, pH 6.65; triangles, pH 4.82; circles, pH 8.50. The solid marks refer to experiments at atmospheric pressure, the open marks to experiments at a pressure of 10,000 lb. per square inch.

are represented by a single curve. Each curve shows a rapid initial fall, followed by a slower decrease in activity, in this respect resembling the denaturation of tetanus antitoxin at 65°C.<sup>10</sup> and diphtheria antitoxin by urea at room temperature.<sup>11, 12</sup> At 10,000 lb. per square inch the curve has the same general shape as at normal pressure, but the rate, especially in the initial period of rapid denaturation, is considerably less. The results obtained in a few experiments at intermediate pressures fell between the data for atmospheric pressure and the data for 10,000 lb. At lower pH



values in phosphate and in acetate buffer (Fig. 1) the antibody is more stable, and the pressure effects appear to be less marked.

It was demonstrated that the products of denaturation do not affect the titre, as shown by control experiments in which mixtures of largely denatured antibody and untreated antibody were assayed. Thus the apparent decrease in specific reaction rate is not due to such a possible error in titration. For samples heated at pH 8.50 for 150 minutes the per cent denatured is independent of the initial antibody concentration over a range from three times as much to one-third as much as that ordinarily employed, indicating that, despite the apparent complexity of the denaturation reaction, it is a first order process with respect to the antibody. The same amount of inactivation occurred in specimens heated for the same total length of time whether heated continuously or for several periods between which the specimens were cooled to room temperature. Although inspection of the data demonstrates clearly that pressure decreases the rate of inactivation of the antibody, a quantitative expression of this effect is rather difficult to formulate, because of the complexities of the denaturation reaction and the pressure effect on it. The data at pH 8.50 appear to warrant quantitative treatment, however, such as given in the next paragraph.

Since the effect of pressure was not constant during the reaction but greater during the early stages, it was impossible to evaluate the effect of pressure simply by graphic transposition of the data along a logarithmic time plot; the curves could not be brought into coincidence by this treatment. The decrease in specific reaction rate with time could be accounted for either by heterogeneity of the antibody groups or by simultaneous reactions with different rate constants and volume changes. The latter might involve an equilibrium between a native and a "protected" form of the antibody, as has been postulated for the urea denaturation.<sup>12</sup> This mechanism requires that the activity equal the sum of two exponential expressions. This requirement was satisfied by the present data within the experimental error, but since the data are not extensive enough for a really satisfactory test of adherence to this expression, the volume changes have been calculated simply by comparison of the slopes of the two denaturation curves at equal activities of antibody. By this method the apparent volume change of activation was found to be 39 ml. per mole between 100 and 70% activity, 24 ml. per mole in the neighborhood of 40% and 16 ml. per mole at 20%. Interpretation of the change in  $\Delta V^\ddagger$  is difficult at present; it is possible that the over-all reaction may involve at the start a larger portion of each molecule than later on.

In direction and magnitude the effects of pressure are similar in anti-toxin inactivation and in certain protein denaturations.<sup>3-7</sup> Additional evidence is thus provided of significant features common to antibody and

protein denaturations.<sup>11, 12</sup> Further studies on the influence of pressure in relation to temperature, pH and denaturants should considerably aid in understanding the mechanism of the reactions.

*Summary.*—At 65°C. denaturation of antitoxin against staphylococcus hemolysin occurs rapidly during the initial part of the reaction. The specific rate decreases as the reaction proceeds, but appears to be independent of the initial concentration of antibody. Hydrostatic pressure (up to 10,000 lb. per square inch) retards the velocity of denaturation; this fact shows that there is a volume increase of the molecules in going from the normal to the activated state. Pressure exerts a greater influence on the reaction during the initial stages of the reaction than during the later stages, and the apparent volume increase of activation changes from 39 ml. per mole during the first 30% of inactivation to 16 ml. per mole after 80% of the antibody has been inactivated.

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## A COMPARISON BETWEEN THE POSITION EFFECTS OF NORMAL AND MUTANT ALLELES

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It has been reported recently<sup>1</sup> that the effect of the recessive mutant allele *cubitus interruptus* (*ci*), located in the small fourth chromosome of *Drosophila melanogaster*, is frequently changed when a rearrangement involving the fourth chromosome has been produced. While the heterozygote between the mutant *ci* and a normal allele  $+$  is nearly always normal when no rearrangement is present, many of the heterozygotes between a "position allele,"<sup>2</sup>  $R(ci)$ , and a normal allele show interruptions of the cubital veins. A comparison of the degree of interruption in the heterozygotes  $R(ci)/+$  with that reported by Dubinin and Sidorov as the position effect of a normal allele of *ci* in rearranged fourth chromosomes of flies with the constitution  $R(+)/ci$ <sup>3, 4</sup> showed that the latter constitution seemed to produce more extreme interruptions than the former. Since, however, the strength of the position effects varies with different rearrangements both for  $R(+)$  and  $R(ci)$  position alleles, and since the genetic background of the  $R(+)$  stocks used by the Russian investigators was presumably different from that present in our  $R(ci)$  stocks, a study of the  $R(+)$  and  $R(ci)$  effect on comparable material was indicated. This paper reports the results of such a comparison.

*Heterozygotes of Position Alleles  $R(ci)$  and a Normal Allele:  $R(ci)/+$ .*—Fifty-five translocations between a fourth chromosome carrying *ci* and one or more of the chromosomes Y, 2 or 3 were discovered, in the offspring of irradiated males which had been made isogenic except for chromosome 4 with an isogenic wild-type stock, Canton-S. Three of these translocations had been lost before the present investigation was started. The remaining fifty-two were tested for the presence of a position effect in heterozygotes with a normal allele of *ci* called  $+$ <sup>2</sup>, present in a stock homozygous for the eyeless-allele *ey*<sup>2</sup>. This  $+$ <sup>2</sup>*ey*<sup>2</sup> stock likewise had been made isogenic with the Canton-S stock, except for chromosome 4. Since it had been found that at 26°C. not only nearly all heterozygotes *ci*/ $+$  but also most  $R(ci)/+$  flies are normal, and since it was known that the lower temperatures enhance the expression of the *ci* phenotype, the cultures were raised at approximately 17°C. Heterozygotes of constitution *ci*/ $+$ , which do not show the position effect, were reared as sibs of  $R(ci)/+$ , and served as controls. With few exceptions, at least three and sometimes more than ten cultures of the parental genotypes  $+$ <sup>2</sup>*ey*<sup>2</sup>/ $+$ <sup>2</sup>*ey*<sup>2</sup> females by single

$R(ci)/ci\ ey^R$  male were raised for each position allele. Such crosses yielded round-eyed  $R(ci)+^{ey}/+^{ey^2}$ , and small-eyed  $ci\ ey^R/+^{ey^2}$  offspring.<sup>5</sup> These were classified individually as to degree of interruption in the section between the posterior crossvein and the wing margin of the cubital vein. Originally, the same classification was used as reported in previous papers, in which normally uninterrupted veins were denoted as *N*, uninterrupted but partially thinned veins as 0, interruption of less than one-half of the vein section as 1, of more than one-half as 2, and complete, or nearly complete, absence of the whole section as 3. For purposes of comparison a mean for the degree of interruption of a group of flies was calculated after giving arbitrary values 0, 1, 2, 3 and 4 to the five phenotypic classes *N*, 0, 1, 2 and 3. This latter classification will herewith be abandoned and be replaced by the classification 0 (formerly *N*), 1 (formerly 0), 2 (formerly 1), 3 (formerly 2) and 4 (formerly 3). While the change in nomenclature may be conducive to some confusion, it is believed that it will simplify the presentation of data to be reported in this and future articles. Females and males will be treated separately because of different degrees of interruption of like genotypes in the two sexes.

The control females  $ci/+^2$  were overwhelmingly (9520 individuals) normal, i.e., of Class 0, with 34 of Class 1 and no fly more extreme than Class 1. This gives a mean value of 0.0036 for expressivity. Of 10,235 control males, 10,097 were of Class 0, 134 of Class 1, and 4 of Class 2, giving a mean of 0.0139. Information on the mean values for each of the  $R(ci)/+^2$  heterozygotes is summarized in lines 1 and 3 of table 1.

TABLE 1

EXPRESSIVITY OF 52 DIFFERENT  $R(ci)$  ALLELES IN HETEROZYGOTES  $R(ci)/+^2\ ey^2$  (17°C.) AND 19 DIFFERENT  $R(+)$  ALLELES IN HETEROZYGOTES  $R(+)/ci\ ey^R$  (26°C.). EACH ENTRY REPRESENTS THE NUMBER OF ALLELES GIVING A MEAN VALUE OF EXPRESSIVITY WITHIN THE RANGE INDICATED

	0.00- 0.09	0.10- 0.49	0.50- 0.99	1.00- 1.49	1.50- 1.99	2.00- 2.49	2.50- 2.99	3.00- 3.49	3.50- 4.00
$R(ci)/+^2\ ey^2\ \text{♀}\ \text{♀}\ *$	25	9	6	3	2	4	2	..	..
$R(+)/ci\ ey^R\ \text{♀}\ \text{♀}$	..	..	..	5	5	6	1	2	..
$R(ci)/+^2\ ey^2\ \text{♂}\ \text{♂}$	23	6	8	4	5	4	2	..	..
$R(+)/ci\ ey^R\ \text{♂}\ \text{♂}$	..	..	1	1	8	4	3	1	1

\* One  $R(ci)$  allele could not be introduced into females.

The distributions of the different  $R(ci)$  alleles should not be taken as final in detail. The errors attached to the individual values have not been calculated but would undoubtedly not exclude slight shifts in the distributions. Moreover, in a few cases the different cultures of a given  $R(ci)$  allele were significantly different in mean values. The causes of such differences are not known except for the probability that an extra fourth chromosome, containing  $ci\ ey^R$  was present in some parents. This would lead to a triplo-

IV condition in part of the offspring and result in a shift of venation types toward normality.<sup>6</sup> In general, each mean value for  $R(ci)/+$  flies entered in table 1 is based on the total distribution of flies from all cultures. It is seen that the expression of the phenotypes of the fifty-two different  $R(ci)/+$  groups varies, with two exceptions in each sex, between the mean value 0 (all wings normal) and less than 2.50 (wing interruption approximately one-half of relevant vein section). The four mean values which are more extreme are only slightly so; the two values for females both being 2.50, and the two values for males being 2.51 and 2.64, respectively.

*Heterozygotes of Position Alleles  $R(+)$  and the Allele  $ci$ :  $R(+)/ci$ .*—In order to obtain  $R(+)$  alleles males of a Canton-S stock were x-rayed. This stock was isogenic except for chromosome 4 with the  $ci$  stock from which the  $R(ci)$  alleles were derived. The x-ray dosage was 4000 r. The irradiated males were mated to non-isogenic females of the constitution attached- $X$   $y$ ;  $ci$   $ey^R$ . The  $F_1$  females and males raised at 26°C. were heterozygous for  $ci$  and were carefully inspected as to the appearance of the cubital vein. Among 1990 females, 17 individuals showed thinning, or interruption of the vein and among 2047 males, 12 such individuals were found. The distribution of these flies over the four abnormal venation classes

	1, 2, 3 and 4
for females was	1, 8, 5 and 3, respectively
and for the males	4, 5, 3 and 0, respectively.

Each of these 29 flies was mated individually to  $ci$   $ey^R$  (isogenic stock). The offspring of the fertile cultures consisted of round-eyed and eyeless flies. Round-eyed males were selected and back-crossed for about ten generations to  $ci$   $ey^R$  flies from stock, in order to approach isogeneity with the  $ci$   $ey^R$  stock except for the irradiated fourth chromosome carrying the wild allele  $+^C$  from Canton-S. This procedure was not possible for three of the original twenty-six cultures since the round-eyed males proved to be sterile. A consideration of these cultures derived from one female each of Classes 2, 3 and 4 will be omitted since isogeneity could not be attained. There remained thus twenty-three cultures, derived separately from flies with a  $ci$  phenotype. Four of these cultures, from three original females and one male, all of Class 2, in later generations failed to show  $ci$  phenotypes and linkage tests proved absence of any translocation. The four ancestors of these cultures either were rare phenotypic overlaps of typical heterozygotes  $+^C/ci$  or possibly were gonosomic mosaics<sup>7</sup> in which an  $R(+)$  allele had participated in the formation of the somatic tissues but no rearrangement had entered the nuclei which went to form the gonads.

The expressivity of  $R(+)$  alleles in the heterozygote  $R(+)/ci$  was studied in the nineteen different stocks finally available. No compre-

hensive chromosomal analyses of these stocks are yet at hand but on the basis of Khwostova's cytological analysis<sup>4</sup> of 196 changed  $+$  alleles, all but three of which proved to be rearrangements, it is assumed that the nineteen changed  $+$  alleles of the present study are  $R(+)$  alleles. Mean values of expressivity were calculated for an average of five separate cultures of each  $R(+)$  allele. In most cases the cultures of a given  $R(+)$  allele gave similar values, but in some cases non-homogeneity was apparent. That this was probably due to presence of an extra fourth chromosome carrying  $ci\ ey^R$ , was confirmed genetically and cytologically in a number of individuals. Total mean values were determined from the sum of the distributions of all cultures of each  $R(+)$  allele. These values, for the females, lie between 1.03 and 3.44, for the males between 0.96 and 3.80 (table 1, lines 2 and 4).

*Comparison Between the Position Effects of Heterozygotes  $R(ci)/+$  and  $R(+)/ci$ .*—The data for this comparison are available in table 1. There is no doubt that all values of  $R(+)/ci$  types represent striking position effects, since heterozygotes between  $ci$  and the  $+^C$  allele in its typical position, at 26°C., are nearly always normal, i.e., give a mean of 0.00 in contrast to the lowest observed  $R(+)/ci$  value of 0.96. It is more difficult to determine which of the  $R(ci)/+$  values represent only low values of typical  $ci/+$  heterozygotes raised at 17° and which  $R(ci)/+$  values can be regarded as characterizing true position effects. For purposes of comparison with  $R(+)/ci$  values, it will be conservative to regard only those  $R(ci)$  alleles as giving position effects whose means in both sexes are larger than 0.10. This eliminates for purposes of comparison, all 25  $R(ci)$  alleles giving mean values of less than 0.09 for the females. Twenty-three of these give similarly low values for the males. The other two  $R(ci)$  alleles have mean values, in males, in the range 0.10–0.89. They, together with a third in the same range, not represented among the female heterozygotes, have to be subtracted from the six means for males listed in the range 0.10–0.49. This reduces to twenty-six the number of  $R(ci)$  alleles available for the comparison with  $R(+)$ . The two distributions, in both sexes, are significantly different, indicating a greater mean position effect for  $R(+)$  alleles than for  $R(ci)$  alleles.

Before accepting this conclusion it must be pointed out that the data from the two types of position alleles are not strictly comparable: (1) the normal allele of  $ci$  present in  $R(+)$  is the Canton-S allele  $+^C$  while that used in the heterozygotes with  $R(ci)$  is the  $+^2$  allele; (2) the culture temperature for  $R(ci)/+$  was 17°C., that for  $R(+)/ci$  26°C. Difference (1), between  $+^C$  and  $+^2$ , should act in the direction of making  $R(+^C)/ci$  more extreme than  $R(+^2)/+$ . However, this effect would be very small.<sup>5</sup> On the other hand, an overwhelming effect acting in the opposite direction on the mean values of  $R(+)/ci$  vs.  $R(+^2)/+$  is due to difference (2). In-



deed, at 26°C. most  $R(ci)$  alleles in the combination  $R(ci)/+$  give nearly exclusively normal wings. Thus in experiments at 26°C. involving nine different  $R(ci)$  alleles, the total mean for the females was 0.005 and for the males 0.009 with the maximum individual means being 0.057 and 0.043, respectively. At 17°C. the individual means for these same  $R(ci)$  alleles lie between 0.300 and 2.083 for the females, and between 0.516 and 2.066 for the males. The low temperature actually used in the experiments with  $R(ci)$ , summarized in table 1, serves to separate phenotypically the position effect yielding  $R(ci)$  alleles from the typical  $ci$  allele in its normal position and from those  $R(ci)$  alleles which do not give position effects. Had the experiments with  $R(ci)/+$  flies been conducted at the same high temperature as those with  $R(+)/ci$  flies the difference in the two distributions would have been much more striking. There would have been very little overlap of the two distributions in either sex since all  $R(ci)/+$  values would lie close to 0, that is, to the left of the  $R(+)/ci$  values.

There is, however, another factor which must be considered. All  $R(+)$  alleles found produced position effects whose mean values either approached a minimum value of 1 or were more extreme. Why were no lower values observed? It is obvious that this is at least partly due to the way in which the  $R(+)$  alleles were discovered, namely, as single individuals showing a vein disturbance of the degree class 1, 2, 3 or 4. Had position alleles originated whose mean effect is very low, as, for instance, 0.1, then most individuals carrying such an allele would appear normal and the probability of the single original fly having abnormal venation and therefore being discovered would be small. Even for  $R(+)$  alleles giving mean values of about 1 the probability of overlap with normal is considerable. These considerations make it apparent that the left end of the distribution of  $R(+)/ci$  mean values is selectively depressed. It can, however, hardly be assumed that this effect is strong enough to obliterate the differences between the  $R(+)/ci$  and  $R(ci)/+$  distributions. As stated before, at a temperature of 26°C. most, if not all  $R(ci)/+$  values would have shifted to the left of all present  $R(+)/ci$  values so that only an improbable large number of undiscovered low  $R(+)$  alleles could make the two distributions alike.

While this analysis upholds the statement that  $R(+)$  alleles give a greater mean position effect than  $R(ci)$  alleles, it was decided to add a direct test. Males homozygous for the constitutions  $ci\ ey^R$  and  $+^C$  were irradiated simultaneously with a dose of 4000 r and then crossed to  $+^C$  and  $ci\ ey^R$  females, respectively. Equal numbers of the two reciprocal crosses were made and the  $F_1$  inspected for venation types. In order to exclude any bias the cultures were coded by a third person so that the investigator did not know to which cross any culture belonged before the counts were completed. As a control reciprocal crosses were made of the same type,

but with non-irradiated  $P$  males. In the control cross  $+^C \text{♀} \times ci \text{ey}^R \text{♂}$  among 1557  $\text{♀}$  and 1855  $\text{♂}$  only normal, Class 0 wings were encountered, and in the reciprocal control cross  $ci \text{ey}^R \text{♀} \times +^C \text{♂}$ , among 1675  $\text{♀}$  and 2037  $\text{♂}$ , only a single male with very weak Class 1 venation. The results of the irradiation experiments are strikingly different (table 2). While the great majority of  $F_1$  flies still showed normal

TABLE 2

DISTRIBUTION OF PHENOTYPES OF  $F_1$  INDIVIDUALS FROM CROSSES OF (a)  $+^C \text{♀} \times ci \text{ey}^R \text{♂}$  AND (b)  $ci \text{ey}^R \text{♀} \times +^C \text{♂}$ , 4000 r. 26°C.

$P$	$F_1$	0	1	2	3	4
(a) $+^C \text{♀} \times ci \text{ey}^R \text{♂}$	$\text{♀}$	2328	3	..	..	..
	$\text{♂}$	2311	2	..	..	..
(b) $ci \text{ey}^R \text{♀} \times +^C \text{♂}$	$\text{♀}$	2219	5	9	10	1
	$\text{♂}$	2139	2	13	6	..

venation, five of the  $F_1$  individuals from the irradiated  $ci \text{ey}^R$  males and forty-six from irradiated  $+^C$  males had  $ci$  type venation. These fifty-one individuals represent, with the possible exception of a very few weak  $ci$  types, position alleles. In spite of equal probability in this experiment of discovering  $R(ci)$  and  $R(+)$  alleles the inequality of the actual finding, namely, 5:46, is proof of an intrinsic difference. Moreover, all five  $R(ci)$  alleles appeared as members of the least extreme Class 1 while only seven of the forty-six  $R(+)$  alleles belong to Class 1, the rest being nearly equally distributed over Classes 2 and 3 except for one belonging to Class 4. The experiment bears out the original contention of greater intensity of position effects produced by  $R(+)$  as compared to  $R(ci)$  alleles.

Still additional support for this result comes from a comparison of the differences between the relevant  $R(ci)/+$  mean values reported in table 1 with the mean values for  $ci/ci$  flies raised at similar temperatures, as calculated from earlier data<sup>9</sup> and the differences between the  $R(+)/ci$  values reported in table 1 and the mean values of their  $ci/ci$  sibs. None of the fifty-two values of both sexes for the heterozygotes between  $R(ci)$  and  $+$  is larger, i.e., more extreme than the values for homozygous  $ci/ci$ , while ten of the thirty-eight heterozygotes between  $R(+)$  and  $ci$  give values larger than the corresponding  $ci/ci$  means. This peculiar phenomenon will be discussed in another communication. It may be concluded that in the average, the position effects of  $R(+)$  alleles are stronger than those of  $R(ci)$  alleles.

**Discussion.**—The main result reported in this paper is the fact that in cases of rearrangements the effect of the heterozygote  $+/ci$  differs according to whether the break in the fourth chromosome is present in the homologue carrying the allele  $+$ , or the allele  $ci$ . In the first case, the heterozygote produces a more extreme mean interruption than in the second. In view of the demonstration<sup>6</sup> that both the normal alleles and the

mutant allele *ci* act toward uninterrupted venation, but with different effectiveness, a more extreme interruption indicates a decreased effect in terms of primary genic action. If it is assumed that the new position of a gene involved in a position allele is responsible either for a change in its functioning or in a decrease in amount of available substrate for its activity, then the result of comparison of  $R(+)/ci$  with  $R(ci)/+$  conforms to a reasonable expectation. A rearrangement might well produce a greater impact on the heterozygote  $+/ci$  if a break is close to the  $+$  allele which in a normal position carries the burden of leading to more or less normal phenotypes than if the break is close to the mutant *ci* allele whose effect is small in comparison to that of  $+$ .

Ephrussi and Sutton<sup>10</sup> have recently proposed a special interpretation "which regards position effect as a result of chromosome pairing which causes a modification of stress near to the affected loci." This interpretation, related to an earlier one by Muller,<sup>11</sup> would lead to the expectation of identity in effect of  $R(+)/ci$  and  $R(ci)/+$  since a distortion by pairing forces from one homologous chromosome to the other should act symmetrically on the two alleles regardless whether they lie in the unchanged or rearranged chromosome. Without additional assumptions the hypothesis of pairing stresses is not supported by the finding of different mean effect of  $R(+)/ci$  and of  $R(ci)/+$ .

*Summary.*—In *Drosophila melanogaster* heterozygotes between the mutant gene *ci* (*cubitus interruptus*) and a normal allele  $+^2$  or  $+^C$  are mostly normal in phenotype. If a rearrangement has taken place near to the *ci* locus the heterozygotes show varying degrees of vein interruptions. This position effect is of different strength according to whether the rearrangement involves the chromosome with a normal allele,  $R(+)$ , or its homologue with the mutant allele,  $R(ci)$ . While different position alleles of either type lead to different mean values for degree of expression, the distribution of means for twenty-six types of  $R(ci)/+$  flies is significantly less extreme than that for nineteen types of  $R(+)/ci$  flies. The significance of these results on interpretations of position effects is indicated.

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<sup>1</sup> Stern, C., and Heidenthal, G., these PROCEEDINGS, 30, 197–205 (1944).

<sup>2</sup> The original definition of the term "position allele" as given in the paper cited in footnote 1 is somewhat extended in this paper. Instead of using "the symbol  $R(ci)$  for any rearrangement ( $R$ ) which leads to a position effect of *ci*" we shall rather use the symbols  $R(ci)$  and  $R(+)$  for any rearrangement involving the neighborhood of the *ci* locus regardless as to whether or not a position effect results from such a rearrangement. This extension becomes necessary on account of the practical difficulty of deciding, in

borderline cases, whether or not a changed effect results from a rearrangement (see below in text).

<sup>2</sup> Dubinin, N. P., and Sidorov, B. N., *Biol. Zhurnal*, 3, 307-331 (1934).

<sup>4</sup> Khwostova, W. W., *Bull. Acad. Sci. U. S. S. R. Serie biologique*, pp. 571-574 (1939).

<sup>5</sup> Except in the table and in cases of special emphasis, references to the *ey* alleles will be omitted in the following.

<sup>6</sup> Stern, C., *Genetics*, 28, 441-475 (1943).

<sup>7</sup> Sidky, A. R., *Jour. Genetics*, 39, 265-272 (1940).

<sup>8</sup> Stern, C., and Schaeffer, E. W., these PROCEEDINGS 29, 361-367 (1943).

<sup>9</sup> No data are available for *ci/ci* flies raised at 16°C. Data collected at 18°C. (see paper quoted under reference 6, tables 12 and 13), which should give less extreme means than 16°C. cultures, provide means of 2.68 and 3.11 in two different experiments for females and 3.07 for males. All these figures are larger than those for any *R(ci)/+* value.

<sup>10</sup> Ephrussi, B., and Sutton, E., these PROCEEDINGS, 30, 183-197 (1944).

<sup>11</sup> Muller, H. J., *Summ. Commun. XV int. physiol. Congr. (Leningrad-Mosc.)* pp. 286-289 (1935); and *Proc. 15th Int. Physiol. Congr. (Leningrad-Mosc.)*, pp. 587-589 (1938).

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## LINKAGE IN THE ALBINO CHROMOSOME OF THE RAT

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The first known case of linkage in the rat was discovered by Castle and Wright in 1916.<sup>1</sup> Repulsion in  $F_1$  hybrids between red-eyed yellow (*r*) and pink-eyed yellow (*p*) was demonstrated. Later it was found that both these characters show linkage with albinism (*c*). The common chromosome pair in which these three genes are borne has been designated the albino or first (I) chromosome pair.

Intensive study was subsequently made of the interrelations of these three genes by Castle, Dunn<sup>2</sup> and Wachter.<sup>3</sup> They found that crossing-over occurs more freely in female than in male  $F_1$  hybrids. In the former the cross-over percentage between *c* and *p* was found to be  $21.93 \pm 0.44$ ; between *p* and *r*,  $20.46 \pm 0.92$ ; and between *c* and *r*,  $0.53 \pm 0.78$ . The values found for males were comparable but smaller, viz.,  $18.4 \pm 0.3$ ,  $15.5 \pm 1.0$  and  $0.18 \pm 0.50$ , respectively. Consequently the order of the three genes was unmistakably *c r p*.

A gene for waltzing (*w*) discovered by Dr. Helen Dean King in 1936<sup>4</sup> was shown by King and Castle<sup>5</sup> in 1937 to be linked with albinism with about 45 per cent of crossing-over. The exact figures were  $44.7 \pm 0.7$  based on the raw data,  $45.8 \pm 0.7$  based on the data corrected for lack of penetrance of waltzing. Since the gene for waltzing (*w*) was obviously more distant



from  $c$  than either  $r$  or  $p$ , it was assumed that the order of the genes was  $c r p w$ .

To test this assumption further and in particular to ascertain whether linkage would (as expected) be shown to exist between  $p$  and  $w$ , a three-point cross was made involving the three genes  $c$ ,  $p$  and  $w$ .

The results of this cross were reported by Castle and King in 1941<sup>6</sup> (correction being made for low penetrance of waltzing) as showing, (1) a percentage of crossovers between  $c$  and  $w$  of 42.6, which is in fair agreement with the earlier reported  $45 \pm$ ; (2) a percentage of crossovers between  $c$  and  $p$  of  $22.9 \pm 1.2$ , which is in fair agreement with the earlier finding of Castle and Wachter, of  $21.93 \pm 0.44$ ; (3) a percentage of crossovers between  $p$  and  $w$  of 35.3. It was concluded that chromosome I of the rat contains five recessive genes in the order  $l c r p w$ ,  $l$  standing for the lethal shown by Grüneberg to lie to the left of  $c$  at a distance of 3.3 units.

But Whittinghill (1944)<sup>7</sup> upon subsequent examination of the data challenged our conclusions both as to the existence of linkage between  $c$  and  $w$ , and as to the existence of linkage between  $p$  and  $w$ . He showed that while we had made allowance in our report on the three-point cross for lack of penetrance of  $w$ , we had failed to make allowance for double crossing-over. His argument was based on the gene sequence which we had assumed,  $c p w$ .

In view of Whittinghill's criticism, a recalculation (Castle, 1944)<sup>8</sup> was made of the data from the three-point cross, allowance being made for double crossing-over as well as for lack of penetrance of  $w$ . The conclusion was reached that the crossover percentage between  $p$  and  $w$  was 32.2 on the uncorrected data, but 45.4 on the corrected data. If this latter value were accepted as correct, it would indicate that  $w$  was even more remote from  $p$  than it was from  $c$ , the distance  $p w$  being 45.4, whereas  $c w$  was on the evidence of the three-point cross  $40.6 \pm 1.4$  and on the evidence of earlier and more extensive experiments about 45.

My colleague, Dr. E. R. Dempster, having read the discussion, suggested that  $c$  might be located *between*  $p$  and  $w$ , contrary to the order assumed in our discussion to be  $c p w$ . The same suggestion was made independently by Professor L. L. Burlingame of Stanford University, who has worked out the statistical problem in detail. I am deeply grateful for his kindness in giving me the benefit of his study of the data.

If we assume the order of the genes in the three-point cross to be  $p c w$ , as suggested by Drs. Dempster and Burlingame, the constitution of the

$F_1$  animals employed in the test backcross was  $\frac{p C W}{P c w}$ , their gametes would

be, as indicated in table 1, of eight possible sorts. The numbers of young resulting in each back-cross class are shown for simplicity only in their

corrected form, i.e., with allowance made for low penetrance of waltzing. The raw data are given in the earlier papers of 1941 and 1944.

From table 1 it will be seen that the crossing-over in region 1 (between  $p$  and  $c$ ) indicated by the colored classes is  $35.8 + 24.2 = 60.0$ , which is  $21.3 \pm 2.0\%$  of the colored population, 282. This value 21.3 agrees well with the value for crossing-over between  $p$  and  $c$  obtained by Castle and Wachter, viz.,  $21.9 \pm 0.4$ .

For region 2 (between  $c$  and  $w$ ) the indicated crossovers are  $92.4 + 24.2 = 116.6$ , which is  $41.3 \pm 2.0\%$  of the colored population. This agrees fairly well with the earlier estimates of crossing-over between  $c$  and  $w$ , as being about 45%. It establishes beyond question the existence of linkage between albinism and waltzing.

TABLE 1

RESULTS OF THE TEST CROSS  $F_1 \frac{p C W}{P c w} \times p c w$

GAMETES OF $F_1$	CROSSEOVERS IN REGION	GRAY	YELLOW	ALBINO
$p C W$	0	...	129.6 non-waltzers	...
$P c w$	0	...	...	(145.2) waltzers
$P C W$	1	35.8 non-waltzers	...	...
$p c w$	1	...	...	(145.2) waltzers
$p C w$	2	...	92.4 waltzers	...
$P c W$	2	...	...	(95.8) non-waltzers
$P C w$	1 and 2	24.2 waltzers	...	...
$p c W$	1 and 2	...	...	(95.8) non-waltzers
Totals		60.0	222.0	241.0

The total amount of crossing-over between  $p$  and  $w$  indicated by the colored classes in table 1, if the  $c$  locus is left out of consideration, would be  $45.4 \pm 2.0\%$ . This is a fairly significant indication of linkage between pink-eyed yellow ( $p$ ) and waltzing ( $w$ ), which was a prime objective of the investigation.

If now we accept the conclusion that  $c$  lies between  $p$  and  $w$ , it becomes necessary to reconsider the position of two other genes,  $r$  and  $l$  in the chromosome I map. It is known that  $r$  lies between  $p$  and  $c$ , very close to the latter. Grüneberg has reported that  $l$  (a lethal) lies at 3.3 units from  $c$  on the side away from  $p$ . This would place it between  $c$  and  $w$ . Consequently the map will become on the best evidence at present available (based primarily on crossing-over in females):

$$\begin{array}{ccccccc} p & 20.5 & r & 0.5 & c & 3.3 & l & 42? & w \\ \hline & 0 & & & & & & & 66.3 \end{array}$$

- <sup>1</sup> Castle, W. E., and Wright, S., *Carnegie Inst. Wash. Publ.*, 241, 175 (1916); 288, 29 (1919).  
<sup>2</sup> Dunn, L. C., *Genetics*, 5, 325 (1920).  
<sup>3</sup> Castle, W. E., and Wachter, W. L., *Genetics*, 9, 1 (1924).  
<sup>4</sup> King, H. D., *Jour. Mam.*, 17, 157 (1936).  
<sup>5</sup> King, H. D., and Castle, W. E., these *PROCEEDINGS*, 23, 56 (1937).  
<sup>6</sup> Castle, W. E., and King, H. D., *Ibid.*, 27, 394 (1941).  
<sup>7</sup> Whittinghill, M., *Ibid.*, 30, 221 (1944).  
<sup>8</sup> Castle, W. E., *Ibid.*, 30, 226 (1944).

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## INDUCED MUTATIONS AND POSSIBLE MECHANISMS OF THE TRANSMISSION OF HEREDITY IN *ESCHERICHIA COLI*

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One of the most remarkable facts brought to light by genetics is the fundamental similarity in the most diverse organisms of the basic mechanisms of the transmission of heredity from parents to offspring. The principal carriers of heredity appear generally to be discrete corpuscles, genes. Only in bacteria and in other asexual lower organisms is the nature of the hereditary transmission still unclear. The most efficient method in use for the detection of genes—namely, the crossing of different strains and observation of segregations in the offspring of the hybrids—is not applicable to asexual forms. The problem must be approached, then, with the aid of the less direct, yet in the final analysis reliable, method of studying mutations, particularly those induced by irradiation. As materials for investigations on the induction of mutations by radiation, bacteria have, in fact, certain advantages not found in higher organisms.

Notwithstanding the large amount of work that has been done in radiation genetics, the events that lead to the manifestation of changes in genes in irradiated cells are not well understood. One important reason for this situation is that in the materials most studied (*Drosophila*, maize) the effects of irradiation cannot be determined until after a considerable number of cell divisions have gone by following the treatment. Since in bacteria it is possible to observe induced changes soon after they occur, the work to be described here was undertaken in the hope that the results might contribute toward a better understanding of the origin of these changes.

It is known from the work of Luria and Delbrück<sup>1</sup> and of Demerec and Fano<sup>2</sup> that in the *B* strain of *Escherichia coli* spontaneous mutations to resistance to the bacteriophage now known as T1 occur with a frequency of about  $1 \times 10^{-8}$  per generation, and that at least two types of such mutants can be recognized. The more common type forms large colonies,

while the other forms tiny colonies. In this study the over-all mutability of *B* to resistance to T1 (*B*/1) was investigated without separating the two types of mutants.

**Materials.**—The mutant strain known as *B*/*r* (*B* resistant to radiations), which came from the *B* strain of *E. coli*, was used in these experiments. This mutant, found and studied by Mrs. Witkin,<sup>3</sup> was selected for our work because it is more resistant than the original *B* strain to both ultra-violet and x-ray irradiation. Since it can stand treatment with higher dosages of both radiations than the *B* strain, it was hoped that increases in the mutation rate would be more easily detectable by irradiating *B*/*r*. This strain was used in all experiments mentioned in this paper. For reasons of convenience, the abbreviated symbol, *B*, instead of the full symbol *B*/*r* will be used here.

Bacteriophage T1, known also as alpha and as P28, was used to isolate mutants resistant to that phage.

**Methods.**—The standard method used in this laboratory for determining how many bacteria resistant to a certain phage are present in a population of sensitive bacteria is, first, to plate on nutrient agar in a Petri dish about  $5 \times 10^8$  phage particles and, a few minutes later, to plate on the same dish the bacteria to be tested. When this procedure is followed the phage comes into contact with the bacteria and lyses all those that are sensitive to the particular phage used, while the resistant ones survive to form colonies. By counting these colonies, the number of resistant bacteria among the total number plated is determined.

In our experiments it proved necessary to detect not only the number of *B*/1 mutants (bacteria resistant to T1 phage) that showed up before the bacteria had had a chance to divide, but also the number that appeared after the bacteria had passed through a certain number of divisions. It was not satisfactory to let the bacteria divide in broth and then determine the number of *B*/1 individuals by the standard method, because after several divisions it would have been impossible to distinguish between *B*/1 bacteria that had originated as mutations and those that had resulted from division of *B*/1 mutants present in the culture as a result of mutations occurring during one of the earlier divisions.

An effective method was developed, which satisfies the requirements of our experiments. A known number of bacteria is plated on nutrient agar medium on Petri dishes. These are incubated for the period of time necessary for the bacteria to pass through the desired number of divisions, and then the phage is sprayed over the surface of the plates as a fine aerosol. For this purpose we use a De Vilbiss glass nebulizer number 44. A stock suspension, containing about  $5 \times 10^9$  phage particles per milliliter, is placed in the chamber of the nebulizer and the aerosol is generated by an air flow of six liters per minute, regulated by a Linde Oxygen Therapy

Station Flow Meter. Each plate is exposed to the phage aerosol for 1 minute by holding it close to the mouth of the nebulizer and moving it around during the exposure so that all regions of the plate are uniformly covered. Tests have shown that when this procedure is followed a sufficient number of phage particles adhere to the plate to produce complete lysis of sensitive bacteria, while resistant bacteria continue to divide and form colonies. The main advantage of this method is that the positions of the bacteria in a growing colony are not disturbed by application of the phage, and that therefore all mutations occurring within a certain period are represented by single colonies, no matter how many mutant bacteria have been formed by division of the original mutant individual. During the development of this method, tests were made of 201 colonies that appeared on a number of plates after exposure to T1 aerosol. All were found to be resistant to T1 and sensitive to another phage active on *B* (T2), indicating that the exposure to the phage had been sufficient to eliminate all sensitive bacteria without introducing any contaminant.

When this aerosol method for applying phage is used, it is important to take precautions against contaminating the laboratory with the phage. If spraying is done in the open the aerosol spreads rapidly from room to room and the phage may persist for several days. To prevent this, spraying is done in a box, measuring  $25 \times 25 \times 23$  cm., with a glass top, an opening on one end for the nebulizer, an opening on the other end with a cloth sleeve through which the arm holding a Petri dish is inserted, and an opening on one of the two sides with a tube through which air is sucked out of the box. During the operation, air containing the phage aerosol is drawn out from the box at a slightly higher rate than it is blown in through the nebulizer, and is passed through a heated copper coil to destroy the phage.

For ultraviolet treatment we used a General Electric germicidal lamp, about 80 per cent of whose output is radiation of wave length 2537 Å. In all the experiments, the material was irradiated at a distance of 92 cm. from the source, where the intensity was approximately 4.2 ergs per second per square millimeter. In a majority of the experiments, 0.05 milliliter of broth containing bacteria was spread over a surface about 8 cm. in diameter in a flat Petri dish 10 cm. in diameter, so that the walls of the dish would not shade the bacteria. After exposure, the material was washed out with a known quantity of broth and used in the experiments.

X-ray treatment was done at the Memorial Hospital in New York City, under the supervision of Miss E. Focht, through the courtesy of Mr. L. D. Marinelli. A bacterial suspension in broth was irradiated in small glass tubes, with unfiltered rays generated at 180 kv. and 25 Ma. at an intensity of 2050 roentgens per minute.

*Experimental Results.—Experiments with ultraviolet:* In the first set of



experiments, we used a 24-hour-old culture of bacteria, which was concentrated by centrifuging so as to contain about  $10^9$  bacteria per milliliter. Samples of this suspension were irradiated in thin layers, then resuspended in suitable amounts of broth to give comparable concentrations of living bacteria in all samples.

The numbers of living bacteria in each sample were determined by plating and colony counts. The original suspension was also tested for number of *B/1* mutants. When exceptionally high numbers of mutants were present in the original culture, they would mask any increase in mutation rate produced by the radiation; and therefore experiments in which this happened were not taken into account.

From each of the irradiated and control samples, twelve portions of 0.1 ml. were plated on different plates. Three plates from each sample were phage treated immediately, three were treated after 2 hours of incubation, three after 3 hours, and the last three after 4 hours. These plates were then incubated, and counts of phage-resistant colonies were made after 48 hours.

TABLE 1

SUMMARY OF 7 EXPERIMENTS, SHOWING THE NUMBER OF *B/1* MUTANTS AMONG *B* BACTERIA TREATED WITH VARIOUS DOSAGES OF ULTRAVIOLET AND INCUBATED FOR VARIOUS LENGTHS OF TIME BEFORE APPLICATION OF PHAGE

INCUBATED BEFORE PHAGING	NUMBER OF <i>B/1</i> COLONIES AFTER IRRADIATION OF			CONTROL
	4 MIN.	2 MIN.	1 MIN.	
0	19	6	4	0
2 hrs.	118	95	24	4
3 hrs.	548	414	226	55
4 hrs.	2081	1234	486	295
No. of bacteria plated	$1.12 \times 10^7$	$1.23 \times 10^7$	$8.61 \times 10^6$	$2.42 \times 10^7$

To determine the growth rate of bacteria after irradiation, dilutions from each sample were also incubated in broth and plated at intervals. From these platings, the number of bacteria present on the plates at various times was calculated, on the assumption of similar rates of growth on agar and in broth.

With minor variations, the procedure outlined above was used in the seven experiments summarized in table 1. This table shows the total number of bacteria plated in each series and the numbers of *B/1* colonies found on Petri dishes where the phage had been added immediately after irradiation or after incubation periods of 2, 3 or 4 hours. It is evident that in all tests made with treated series the numbers of *B/1* colonies were considerably higher than in the control series.

The larger number of *B/1* in the series where the phage was applied immediately after irradiation indicates that the ultraviolet treatment was effective in inducing *B/1* mutants which appeared before the first bacterial division had been completed.

TABLE 2  
ANALYSIS OF DATA FROM TABLE 1, GIVING ESTIMATES OF THE MUTATION RATES

INCUBATED BEFORE PHAGING	4 MIN.			2 MIN.			1 MIN.			CONTROL		
	NO. OF BACTERIA	B/1	RATE $\times 10^8$	NO. OF BACTERIA	B/1	RATE $\times 10^8$	NO. OF BACTERIA	B/1	RATE $\times 10^8$	NO. OF BACTERIA	B/1	RATE $\times 10^8$
0	1.12 $\times 10^7$	19	170	1.23 $\times 10^7$	6	49	8.61 $\times 10^6$	4	46	2.42 $\times 10^7$	0	...
2 hrs.	1.79 $\times 10^7$	118	...	2.71 $\times 10^7$	95	...	3.79 $\times 10^7$	4	...	2.4 $\times 10^8$	4	...
Increment 0-2 hrs.	0.67 $\times 10^7$	99	1478	1.48 $\times 10^7$	89	601	2.93 $\times 10^7$	20	68	2.2 $\times 10^8$	4	1.8
3 hrs.	1.43 $\times 10^8$	548	...	2.17 $\times 10^8$	414	...	3.03 $\times 10^8$	226	...	19.2 $\times 10^8$	55	...
Increment 2-3 hrs.	1.25 $\times 10^8$	430	344	1.90 $\times 10^8$	319	168	2.65 $\times 10^8$	202	76	16.8 $\times 10^8$	51	3.0
4 hrs.	11.44 $\times 10^8$	2081	...	17.36 $\times 10^8$	1234	...	24.24 $\times 10^8$	486	...	153.6 $\times 10^8$	295	...
Increment 3-4 hrs.	10.01 $\times 10^8$	1533	153	15.19 $\times 10^8$	820	54	21.21 $\times 10^8$	260	12	134.4 $\times 10^8$	240	1.8

Growth tests made in broth showed that the initial period of lag in the growth (lag period) is lengthened in treated bacteria, an observation which has already been made by Hollaender and Duggar.<sup>4</sup> After an incubation period of 2 hours, the number of bacteria in the control series increased on the average by a factor of 10, the number in material treated for one minute increased 4.4 times, that in material treated 2 minutes 2.2 times, and that in material treated 4 minutes 1.6 times. After the lag period had passed, both treated and control bacteria multiplied regularly, doubling their number once in about every 20 minutes. No study was made of the multiplication of bacteria on agar, but it is unlikely that there is any considerable difference between the rates of growth on agar and in broth during the several early divisions before the number is large enough to produce crowding on agar. Indeed, observations made under the microscope indicate that the length of the lag phase on agar is about the same as that in broth.

Thus when the phage was applied to cultures that had been incubated for 2 hours in the series that received 4-minute irradiation the bacteria were just passing through the first division, while in the other three series they had already passed through one, two, and more than three divisions, respectively, by the time the phage was applied. Table 1 shows that the number of *B/1* mutants, where plates were incubated for 2 hours before adding phage, was very much greater than in the controls. A similar situation obtained in the case of cultures that were incubated for 3 and for 4 hours before phage treatment. It is evident that new *B/1* mutants continued to appear at a higher

rate in the treated series than in the controls, even after the bacteria had passed through several divisions.

From the data given in table 1, and from the calculated number of bacteria present at each time, the rate of mutation of *B* to *B*/1 may be estimated. Such an analysis of the data is presented in table 2. For the cultures where phage was applied immediately after treatment, the mutation rate was calculated directly from the number of bacteria plated and the number of *B*/1 colonies observed. In order to calculate the mutation rate among bacteria that were formed by division of plated individuals during the 2 hours of incubation, the total number of bacteria present after that time had to be estimated. This was done by multiplying the number of plated bacteria by 1.6, 2.2, 4.4 and 10 for the 4-, 2- and 1-minute series and the controls, respectively. As has already been pointed out, these values represent average increases in the number of bacteria and were determined by parallel experiments made in broth.

Since the rate of division of irradiated bacteria becomes normal once they pass the lag phase, and since the lag phase is completed in all series after 2 hours of incubation, it is assumed that from then on the number of individuals in each of the series doubles every 20 minutes. For each one-hour period, then, the number increases by a factor of 8. Consequently, values for the total number of bacteria present after 3 and 4 hours of incubation were obtained by multiplying by 8 the estimated values at the end of the previous hour.

It is realized that these estimates are only approximate. However, it is felt that the approximation is sufficiently close for the purpose at hand.

Examination of table 2 reveals that the calculated rate of mutation of *B* to *B*/1, per number of bacteria, increases in the treated series until the bacteria have passed through one or two divisions. Thereafter the rate begins to fall.

In order to determine if or when the rates reach the normal level of about  $1 \times 10^{-8}$ , a set of six experiments was performed in which bacteria were irradiated for 4 minutes and phage applied either immediately after the treatment or after incubation of 4, 5 or 6 hours. A summary of these experiments is presented in table 3, in the same form as the data shown in table 2. To obtain the estimate of the total number of bacteria present after 4 hours of incubation, the number of plated bacteria was multiplied by 102.4, which is equal to the  $1.6 \times 8 \times 8$  used in table 2. Values for 5 and 6 hours were obtained by multiplying by 8 for each 1-hour period. It is evident from table 3 that the mutation rate continued to decrease with subsequent bacterial division and that at 6 hours, when the bacteria had passed through approximately 13 divisions, it had reached normal level.

*Experiments with x-rays:* A 48-hour culture of bacteria in broth was used as the source of material. About 1.3 cc. of bacterial suspension was



placed in each of three small glass tubes, which were then irradiated with 10,000, 20,000 and 50,000 roentgens, respectively. After irradiation, 15 platings of 0.05 cc. of the bacterial suspension were made on Petri dishes from each of the three tubes. Three Petri dishes from each set were exposed to phage aerosol immediately after plating, and others were exposed in groups of three after incubation of 1, 2, 3 and 4 hours. A summary of two experiments is given in table 4. The data are arranged as in table 2.

Tests made in broth indicated that the lag period of the bacteria was not materially affected by x-ray treatment. Under the conditions of these experiments, the number of bacteria doubled after 1 hour in broth at 37°C.

It is evident from table 4 that, as in the ultraviolet experiments, only a fraction of the induced mutants showed up immediately after treatment, while the appearance of a large proportion of them was delayed until after the treated bacteria had passed through several divisions. Comparisons of the data of tables 2 and 4 indicate obvious differences between the ultraviolet and the x-ray results. These are now being investigated further, and an analysis of this work will be presented in another paper.

TABLE 3

SUMMARY OF 2 EXPERIMENTS, WITH ESTIMATES OF THE RATES OF MUTATION OF *B* TO *B/1* AFTER ULTRAVIOLET TREATMENT OF 4 MINUTES FOLLOWED BY INCUBATION FOR VARIOUS LENGTHS OF TIME BEFORE APPLICATION PHAGE

INCUBATED BEFORE PHAGING	NUMBER OF		RATE × 10 <sup>4</sup>
	BACTERIA	<i>B/1</i>	
0	$2.25 \times 10^8$	7	311.1
4 hrs.	$2.3 \times 10^8$	234	...
Increment 0-4 hrs.	.....	227	101.7
5 hrs.	$18.4 \times 10^8$	297	...
Increment 4-5 hrs.	$16.1 \times 10^8$	63	3.9
6 hrs.	$147.2 \times 10^8$	543	...
Increment 5-6 hrs.	$128.8 \times 10^8$	246	1.9

*Discussion.*—Changes induced by irradiations cannot be distinguished from those which occur spontaneously and which have already been extensively studied.<sup>1, 2</sup> In the course of these irradiation experiments several hundred *B/1* strains were grown in broth and tested for resistance to T1 and T2. They were found to be resistant to the first-mentioned phage and sensitive to the second, just as were the spontaneously originating strains described in previous experiments. Similar morphological types are found among both spontaneous and radiation-induced mutants—namely, a type that forms normal, large colonies and one that forms tiny colonies.

The simplest interpretation of the changes from *B* to *B/1* is that they are mutations comparable to gene mutations in higher organisms. However, since our knowledge of the mechanisms governing the transmission of heredity from parents to offspring in bacteria is still inadequate, other

TABLE 4  
SUMMARY OF 2 EXPERIMENTS, WITH ESTIMATES OF THE RATES OF MUTATION OF *B* TO *B/1* AFTER X-RAY TREATMENT WITH VARIOUS DOSAGES AND INCUBATION FOR VARIOUS LENGTHS OF TIME BEFORE APPLICATION OF PHAGE

INCUBATED BEFORE PHAGING	10,000 r				20,000 r				50,000 r			
	BACTERIA	NO. OF	<i>B/1</i>	RATE × 10 <sup>3</sup>	BACTERIA	NO. OF	<i>B/1</i>	RATE × 10 <sup>3</sup>	BACTERIA	NO. OF	<i>B/1</i>	RATE × 10 <sup>3</sup>
0	7.8 × 10 <sup>7</sup>	21	26.9	...	6.2 × 10 <sup>7</sup>	44	71.0	...	7.2 × 10 <sup>6</sup>	57	791.7	...
1 hr.	1.56 × 10 <sup>8</sup>	57	...	...	1.24 × 10 <sup>8</sup>	51	...	...	1.44 × 10 <sup>7</sup>	88	...	...
Increment 0-1 hr.	0.78 × 10 <sup>8</sup>	36	46.2	...	0.62 × 10 <sup>8</sup>	7	11.3	...	0.72 × 10 <sup>7</sup>	31	430.6	...
2 hrs.	12.5 × 10 <sup>8</sup>	148	...	...	9.9 × 10 <sup>8</sup>	224	...	...	1.2 × 10 <sup>8</sup>	291	...	...
Increment 1-2 hrs.	10.9 × 10 <sup>8</sup>	91	8.3	...	8.7 × 10 <sup>8</sup>	173	19.9	...	1.06 × 10 <sup>8</sup>	203	191.5	...
3 hrs.	100.0 × 10 <sup>8</sup>	318	...	...	79.2 × 10 <sup>8</sup>	353	...	...	9.6 × 10 <sup>8</sup>	378	...	...
Increment 2-3 hrs.	87.5 × 10 <sup>8</sup>	170	1.9	...	69.3 × 10 <sup>8</sup>	129	1.9	...	8.4 × 10 <sup>8</sup>	87	10.3	...
4 hrs.	...	...	...	...	...	...	...	...	76.8 × 10 <sup>8</sup>	569	...	...
Increment 3-4 hrs.	...	...	...	...	...	...	...	...	67.2 × 10 <sup>8</sup>	191	2.8	...

possibilities should be considered. Only by eliminating such alternative explanations can one hope to determine whether the hereditary mechanisms in bacteria are fundamentally similar to those in higher forms.

For example, the genes in bacteria may not be organized into chromosomes, and several or many genes of the same kind may be present in each individual. Recessive mutations—which, as is well known, constitute a large proportion of all mutations—would then attain phenotypic expression only after the individual had passed through several divisions, and the mutant gene had been sorted out and had replaced its allele in some individuals.

Certain other interpretations should be considered. All changes from *B* to *B/1* affect reactions between bacteria and phages. Since sensitive bacteria adsorb phages but resistant ones do not, and since adsorption is a surface phenomenon, it is possible that changes in the surface bring about a condition which ultimately may be responsible for the origin of resistance. It might be supposed that resistance originates as a change in a small sector of bacterial surface, for example, a receptor, and that it spreads in successive divisions so that it finally affects one whole bacterium, which then reproduces as a resistant mutant.

However, the evidence presented here shows that some of the induced mutations become phenotypically effective at the time of the first bacterial division. This would indicate that the mechanism responsible for the transmission of resistance to phage is neither

one involving many genes of the same kind, as first suggested, nor a surface phenomenon which would require several cell divisions before being manifested.

Two points should be particularly emphasized in discussing the data presented in this paper: (1) that some of the induced mutants are phenotypically effective by the time the first bacterial division occurs, and (2) that new mutants continue to appear during several bacterial generations.

It is known from the work of Delbrück<sup>5</sup> that one sensitive bacterium may adsorb as many as 250 phages, while resistant bacteria do not adsorb any. Presumably an adsorbed phage enters a bacterium and multiplies therein until finally the bacterium is lysed—it bursts and liberates phage particles. Therefore, there are two critical steps in the life of a bacterium which determine whether or not it will be lysed by phage if this is present. These steps are the adsorption of phage by the surface and the multiplication of phage within a bacterium. It is evident that if either of these two processes is prevented from occurring a bacterium will become resistant to phage, and that if a mutation in a bacterium is capable of making it resistant it must be capable of blocking one of these two processes. If the adsorption of phage were immediately blocked by a mutated gene, this would mean that such a gene exerted an immediate influence on the organism and affected the forces active in adsorption. On the other hand, if a mutation prevented the multiplication of phage, this might mean that the mutated gene became active during the bacterial division, since, as a rule, the multiplication of phage is intimately connected with the division of bacteria.

Experiments were designed in such a way as to leave very little doubt that *B/1* mutants that appeared after bacteria had passed through several divisions were due either to mutations that originated after irradiation or mutations that were induced at the time of irradiation but failed to manifest their effect until the organism had passed through several cell divisions.

The simplest way to explain the appearance of mutants immediately after treatment as well as after several bacterial divisions would be to assume that these bacteria are diploid, and that the immediate mutants are a result of coincidental changes of both alleles by two independent hits, while the delayed mutants appear as a result of segregation of heterozygotes. Two objections make this hypothesis improbable. One of them is the large number of immediate mutants found in the ultraviolet and, particularly, the x-ray experiments. The observed numbers of these mutants differed from the calculated values by a factor of about 10 in the ultraviolet experiments and by a factor of about 1000 in the x-ray experiments. These discrepancies could be accounted for by assuming that some of the homozygous mutants could be induced by a single hit—particularly in the case of x-rays. Such an assumption would not be unreasonable, since Lea and Catcheside<sup>6</sup> have estimated that the field of action of one x-ray

ionization is about 0.1 micron, so that any two alleles that happened to be located that close to each other would have a chance of being affected together.

The other, and more serious, objection against this hypothesis is the distribution of mutants. A few of them appear immediately after treatment, but the larger proportion do not express themselves until after several cell divisions have taken place. If delayed mutants were due either to a simple segregation of a heterozygote or to aberrant divisions of asexual cells resulting in segregations, it would be expected that the largest proportion of delayed mutants would appear during the second bacterial generation, and that the frequency would rapidly diminish thereafter.

Another possible explanation of the observed delay in the appearance of mutants assumes that the bacterial population used in experiments was not homogeneous—that is, that some bacteria were haploid, others diploid, and still others polyploid. By assuming certain proportions of the various types, a mixture could be postulated which would give the observed results. In all the experiments reported so far, resting bacteria were used; these were obtained by growing bacteria in broth, at 37°C., for either 24 or 48 hours. It is known that growing bacteria are physiologically very different from resting bacteria. Since the proportions of haploid, diploid and polyploid individuals—if these did occur at all—might be expected to be different in different physiological conditions, we compared data obtained from ultraviolet irradiation of resting and actively growing bacteria. The results were not significantly different.

Another possible hypothesis assumes that all mutations occur during irradiation but that some are delayed in their manifestation until the supply of the substrate manufactured by the gene and necessary for the production of material that makes the bacteria sensitive to the phage is exhausted. It might then be expected that changes in the environment might affect the rate of utilization of the substrate.

Still another hypothesis may be considered to explain the results obtained. It may be assumed that two types of change are induced by irradiation—one type producing gene changes, and the other inducing some change in the cell, either of the chromosomes or of the cytoplasm, which increases the mutability of the gene system. The latter type of change would decrease in effectiveness with each cell division, and after a number of divisions lose its potency. According to this hypothesis, the calculated mutation rate per number of bacteria would be highest after the early divisions, a situation observed in our experiments (table 2).

In irradiation work with *Drosophila* a similar delayed effect is observed after treatment of sperm. In that case mosaics, or "fractional" mutants, are obtained. Muller<sup>7</sup> and several other workers have interpreted this observation by assuming that the chromosomes in some of the sperms are

already split, and that in such cases the irradiation produces a change in only one of the two strands. Neuhaus,<sup>8</sup> however, after studying the distribution of the size of mosaic regions, reached the conclusion that these mosaics are due to new mutations occurring as a result of a delayed effect of the irradiation.

In the experiments described in this paper various doses of ultraviolet radiation and x-rays were used. The data indicate an increase in the effect with increase of the dosage. Specially designed experiments are now under way, however, to determine the relationship between the dosage and the number of induced mutants; this problem will be discussed elsewhere. An effort is being made also to devise experiments which will differentiate between the various possibilities for interpretation of the results of experiments discussed here.

*Summary.*—The rate of mutation from sensitivity to resistance to bacteriophage T1 in the *B* strain of *E. coli* was measured after treatment with ultraviolet radiation (2537 Å) and with x-rays.

Both radiations produced a substantial increase in the mutation rate, which increase became greater with higher doses of radiation.

Some of the induced mutations manifest themselves by the time the treated bacteria begin to multiply, while the majority of them express themselves after the bacteria have passed through several divisions.

Several possibilities for interpretation of results are discussed.

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# ON THE NUMBER OF SOLUTIONS OF SOME GENERAL TYPES OF EQUATIONS IN A FINITE FIELD

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Consider the equation

$$c_1x_1^{a_1} + c_2x_2^{a_2} + \dots + c_sx_s^{a_s} + c_{s+1} = 0 \quad (1)$$

in the  $x$ 's, where the  $a$ 's are integers such that  $0 < a \leq p^n - 1$ ;  $s \geq 2$ ; the  $c$ 's belong to a finite field of order  $p^n$ ,  $p$  prime, which will be designated by  $F(p^n)$ ; and

$$c_1c_2 \dots c_sx_1x_2 \dots x_s \neq 0$$

in  $F(p^n)$ . The problem discussed here will be that of *determining directly* the number of solutions  $x_1^{a_1}, x_2^{a_2}, \dots, x_s^{a_s}$  in  $F(p^n)$  of (1). By "determining directly" we shall mean the determination of this number in a finite number of steps without trial. When  $n = 1$  it is possible to carry this out using a method given by the writer,<sup>1</sup> but this scheme leads to very complicated procedures when  $s > 2$  in (1). In the present paper we shall employ other methods. The main result (Theorem I) established reduces the general problem to the determination of the number of solutions  $y_1^m$  and  $y_2^m$  in

$$cy_1^m + dy_2^m + 1 = 0; \quad y_1y_2 \neq 0$$

for some integer  $m$  depending on the  $a$ 's and  $p^n$ , with  $c$  and  $d$  any given non-zero elements of  $F(p^n)$  *provided a table of indices for  $F(p^n)$  is known*, that is, if  $g$  is a generator of the cyclic group formed by the non-zero elements of  $F(p^n)$  then we know the value of  $z$  in

$$g^z = k; \quad 0 \leq z < p^n - 1$$

for a given  $k$ , and if  $z$  is given we know  $k$ . We write  $z = \text{ind } k$ .

Using indices we first consider (1) with  $c_{s+1} \neq 0$ . After dividing through by this element our equation may be put in the form

$$1 + E(e_1 + \alpha_1a_1) + E(e_2 + \alpha_2a_2) + \dots + E(e_s + \alpha_sa_s) = 0 \quad (2)$$

with  $0 \leq e_i < a_i$ ;  $i = 1, 2, \dots, s$ , and where  $E(t)$  denotes  $g^t$ . In this relation it is sufficient to take the  $\alpha$ 's in the range  $0, 1, 2, \dots, (p^n - 1)/d_i - 1$ , where  $(a_i, p^n - 1) = d_i$ ;  $i = 1, 2, \dots, s$ . Also there is a one-to-one correspondence<sup>2</sup> between a solution  $E(\alpha_1a_1), E(\alpha_2a_2), \dots, E(\alpha_sa_s)$  of (2) and a certain solution  $E(\beta_1d_1), E(\beta_2d_2), \dots, E(\beta_sd_s)$  of

$$1 + \sum_{i=1}^s E(e_i + \beta_i d_i) = 0 \quad (3)$$

where  $\beta_i$  lies in the range

$$0, 1, 2, \dots, (p^n - 1)/d_i - 1; \quad i = 1, 2, \dots, s.$$

By the use of the Euclidean algorithm in connection with the exponents we see that in the consideration of (3) it is sufficient to take each  $e_i < d_i$ .

For any value of  $\beta_1$ , say  $\beta$ , appearing in (3) we may write

$$\beta = w + \gamma \frac{m}{d_1}; \quad 0 \leq w < \frac{m}{d_1},$$

where  $m$  is the L.C.M. of the  $d$ 's in (3). Then

$$e_1 + \beta d_1 = (e_1 + w d_1) + \gamma m$$

and

$$e_1 + w d_1 = e_1 + d_1 \left( \frac{m}{d_1} - 1 \right) = e_1 - d_1 + m < m,$$

since  $d_1 > e_1$ . This  $\beta$  then gives a solution in  $E(\gamma m), E(\beta_2 d_2), \dots, E(\beta_s d_s)$  of the equation

$$E(e_1 + w d_1 + \gamma m) + E(e_2 + \beta_2 d_2) + \dots + E(e_s + \beta_s d_s) + 1 = 0,$$

with  $0 \leq \gamma < (p^n - 1)/m$ .

Conversely any solution in  $E(\delta m), E(\beta_2 d_2), \dots, E(\beta_s d_s)$  of

$$E(e_1 + v d_1 + \delta m) + E(e_2 + \beta_2 d_2) + \dots + 1 = 0$$

for a given  $v$  such that  $0 \leq v < \frac{m}{d_1}$  with  $0 \leq \delta < (p^n - 1)/m$ , gives a solution of (3) for we have

$$e_1 + v d_1 + \delta m = e_1 + d_1 \left( v + \delta \frac{m}{d_1} \right)$$

and it is found that  $v + \delta \frac{m}{d_1}$  is in the range  $0, 1, 2, \dots, ((p^n - 1)/d_1 - 1)$ .

Hence  $v + \delta \frac{m}{d_1}$  is a value for  $\beta_1$  in (3). In view of this we may obtain the number of solutions of (3) in

$$E(\beta_1 d_1), E(\beta_2 d_2), \dots, E(\beta_s d_s)$$

by taking the sum of the numbers of solutions in

$$E(\gamma_1 m), E(\beta_2 d_2), \dots, E(\beta_s d_s) \text{ of each of the equations}$$

$$E(k_1) + E(e_2 + \beta_2 d_2) + E(e_3 + \beta_3 d_3) + \dots + E(e_s + \beta_s d_s) + 1 = 0 \quad (4a)$$

where  $k_1 = e_1 + w_1 d_1 + \gamma_1 m$ , and  $w_1$  ranges over each element of the set  $0, 1, 2, \dots, \left(\frac{m}{d_1} - 1\right)$ , and  $\gamma_1$  is to be determined so that  $0 \leq \gamma_1 < (p^n - 1)/m$ .

We now proceed with the exponent  $e_2 + \beta_2 d_2$  in (4a) as we did with the exponent  $e_1 + \beta_1 d_1$  in (3) and we obtain the result that the number of solutions of (3) in

$$E(\beta_1 a_1), E(\beta_2 a_2), \dots, E(\beta_s a_s)$$

is the sum of the number of solutions in

$$E(\gamma_1 m), E(\gamma_2 m), E(\beta_2 a_2), \dots, E(\beta_s a_s)$$

of each of the equations

$$E(k_1) + E(k_2) + E(e_3 + \beta_3 a_3) + \dots + E(e_s + \beta_s a_s) + 1 = 0; \quad (4b)$$

$$k_2 = e_2 + w_2 d_2 + \gamma_2 m;$$

where  $w_1$  ranges as before in  $k_1$  and  $w_2$  ranges over the set  $0, 1, 2, \dots, \left(\frac{m}{d_2} - 1\right)$ . By proceeding in this manner with the other terms in (4b) we derive the result that the number of solutions of (3) equals

$$\sum_{w_1, w_2, \dots, w_s} (e_1 + w_1 d_1, e_2 + w_2 d_2, \dots, e_s + w_s d_s) \quad (5)$$

where  $w_i$  ranges independently over the set  $0, 1, 2, \dots, \left(\frac{m}{d_i} - 1\right)$ ;  $i = 1, 2, \dots, s$ , and the symbol

$$(f_1, f_2, \dots, f_s)$$

means the number of solutions in

$$E(e_1 m), E(e_2 m), \dots, E(e_s m)$$

of

$$E(f_1 + e_1 m) + E(f_2 + e_2 m) + \dots + E(f_s + e_s m) + 1 = 0.$$

We next proceed to show that the number of solutions of

$$1 + \sum_{i=1}^s E(u_i + m r_i) = 0 \quad (6)$$

for a given  $u_1, u_2, \dots, u_s$  may be obtained directly if we know the number of solutions of an equation of the type

$$1 + E(v_1 + m t_1) + E(v_2 + m t_2) = 0 \quad (7)$$

for a given  $v_1$  and  $v_2$ . For the necessary argument we shall find it con-



venient to introduce the notation  $\theta_u$  for  $E(u + mr)$  so that in place of (6) we have

$$\theta_{u_1} + \theta_{u_2} + \dots + \theta_{u_s} + 1 = 0. \quad (8)$$

In other words  $\theta_{u_i}$  is any quantity in  $F(p^n)$  whose index is congruent to  $u_i$  modulo  $m$ . Denote the number of solutions in  $\theta_{u_1}, \theta_{u_2}, \dots, \theta_{u_s}$  of (6) by  $(u_1, u_2, \dots, u_s)$ . We first note that for a given  $h$  and  $t$

$$\theta_{u_1} + \theta_{u_2} + \dots + \theta_{u_s} + E(h + tm) = 0 \quad (9)$$

reduces, when we divide by the last term on the left, to

$$\theta_{u_1 - h} + \theta_{u_2 - h} + \dots + \theta_{u_s - h} + 1 = 0. \quad (10)$$

Consider (8) for  $s \geq 3$ . To enumerate its solutions we proceed by induction. We assume that  $(u_1, u_2, u_3, \dots, u_{s-1})$  for any integers  $u$  may be determined directly. For  $s = 3$ , this reduces to the assumption that the number of solutions of (7) may be determined directly. We shall then prove the same thing for  $(u_1, u_2, u_3, \dots, u_s)$ . Now (8) may be written in the form

$$(\theta_{u_1} + \theta_{u_2} + \dots + \theta_{u_{s-2}} + 1 - \theta_t) + (\theta_t + \theta_{u_{s-1}} + \theta_{u_s}) = 0.$$

Corresponding to each solution of

$$\theta_{u_1} + \theta_{u_2} + \dots + \theta_{u_{s-2}} + 1 - \theta_t = 0 \quad (11)$$

there must be a solution of

$$\theta_t + \theta_{u_{s-1}} + \theta_{u_s} = 0$$

in order that (8) may be satisfied. Using (9) and (10) the number of solutions of the latter is  $(u_{s-1} - i, u_s - i)$  for a fixed  $i$ . Hence the number of solutions of (8) is

$$\sum_{i=1}^{m-1} (i + \beta, u_1, u_2, \dots, u_{s-2})(u_{s-1} - i, u_s - i); \quad (12)$$

$\beta = \text{ind}(-1)$ ; except that in (11)  $\theta_{u_1}, \theta_{u_2}, \dots, \theta_{u_{s-2}}$  might be such that

$$\theta_{u_1} + \theta_{u_2} + \dots + \theta_{u_{s-2}} + 1 = 0 \quad (13)$$

and since this is impossible in (9) with  $\theta_t \neq 0$ , then this case was not included in our enumeration. The relation (13) used in (8) gives

$$\theta_{u_{s-1}} + \theta_{u_s} = 0 \quad (14)$$

so that when this is satisfied we have in place of (12)

$$\sum_{i=0}^{m-1} (i + \beta, u_1, \dots, u_{s-2})(u_{s-1} - i, u_s - i) + \frac{p^n - 1}{m} (u_1, u_2, \dots, u_{s-2}), \quad (15)$$

since it may be shown by known methods that there are  $(p^n - 1)/m$  solutions of (14). By our assumption each term in (12) as well as (15) may be determined directly so either (12) or (15) is fully determined. Hence our induction is complete.

To obtain (2) we assumed that  $c_{s+1} \neq 0$ . The case where  $c_{s+1} = 0$  does not reduce immediately to the case where we have a term unity in our equation as in (2) when it happens that the  $a$ 's are not all equal. Hence we now consider this second case. But if we proceed as we did with (2) we find that such an equation reduces to the solution of an equation of the type

$$E(b_1 + m\sigma_1) + E(b_2 + m\sigma_2) + \dots + E(b_s + m\sigma_s) = 0$$

and this reduces to the form (6) when we divide by the last term. Hence we have the

**THEOREM I.** *Let  $g$  be a generator of the cyclic group formed by the non-zero elements of a finite field of order  $p^n$ ,  $p$  prime, and designated by  $F(p^n)$ . Further, let  $a_1, a_2, \dots, a_s$  be a set of positive integers;  $(p^n - 1, a_i) = d_i$ ,  $i = 1, 2, \dots, s$ ; and assume that  $m$  is the L.C.M. of  $d_1, d_2, \dots, d_s$ . Then the number of different solutions in  $F(p^n)$*

$$E(\alpha_1 a_1), E(\alpha_2 a_2), \dots, E(\alpha_s a_s); \quad s > 1;$$

*of the equation*

$$1 + E(e_1 + \alpha_1 a_1) + E(e_2 + \alpha_2 a_2) + \dots + E(e_s + \alpha_s a_s) = 0;$$

$e_i \geq 0$ ; *or of the equation*

$$E(e_1 + \alpha_1 a_1) + E(e_2 + \alpha_2 a_2) + \dots + E(e_s + \alpha_s a_s) = 0$$

*may be determined directly if the number of solutions  $E(\sigma_1 m), E(\sigma_2 m)$  in  $F(p^n)$  of*

$$E(u_1 + \sigma_1 m) + E(u_2 + \sigma_2 m) + 1 = 0$$

*is known for any integers  $u_1$  and  $u_2 \geq 0$ . Here  $E(t) = g^t$ . The determination may be carried out by the use of the formulae (5), (12) and (15).*

**COROLLARY I.** *An equation of the type, with the  $c$ 's given elements of  $F(p^n)$ ,*

$$c_1 x_1^{a_1} + c_2 x_2^{a_2} + \dots + c_s x_s^{a_s} + c_{s+1} = 0$$

*may, by the use of indices, be reduced to one of the forms set out in Theorem I,*

and thus the number of its different solutions,  $x_1^{a_1}, x_2^{a_2}, \dots, x_s^{a_s}$  in  $F(p^n)$  may be directly determined.

When such numbers are found, known methods will readily yield the number of solutions  $x_1, x_2, \dots, x_s$  of the same equation.

In another paper<sup>3</sup> the writer determined the number of solutions in  $F(p)$  of

$$1 + E(k_1 + 4\omega_1) + E(k_2 + 2\omega_2) = 0. \quad (16)$$

The method there employed may be also used to derive the number of solutions of various other trinomial congruences modulo  $p$  (which are isomorphic with equations in a finite field  $F(p)$ ) when the given exponents are small, using the representation of  $p$  by means of various quadratic forms. An example is the equation obtained from (16) by putting 3 in the first exponent instead of 4. By employing Theorem I of the present paper together with some results due mainly to Dickson<sup>4</sup> it is possible to derive similar results. He obtained by the use of quadratic forms explicit expressions for the number of incongruent solutions  $E(m\tau_1), E(m\tau_2)$  of

$$E(l_1 + m\tau_1) + E(l_2 + m\tau_2) + 1 = 0 \quad (17)$$

for  $m = 1, 2, 3, 4, 5, 6, 8, 9, 10, 12$ . By using Theorem I of the present paper and Dickson's formulae just referred to, we obtain the number of solutions of (16) and more generally the number of solutions of (2) whenever  $n = 1$  and the L.C.M. of  $d_1, d_2, \dots, d_s$  in (3) has one of the values given for  $m$  which are listed just below relation (17).

Mitchell<sup>5</sup> obtained arithmetical formulae which give explicitly the number of solutions of (17) in  $F(p^n)$ ,  $n$  even, where an integer  $n_1$  exists such that  $p^{n_1} \equiv -1 \pmod{m}$ . Using these results we may obtain formulae for the number of solutions of (2) whenever the L.C.M. of  $d_1, d_2, \dots, d_s$  is  $m$ , with  $p, n$  and  $m$  related as just stated. These equations may also be applied to (1) without the use of indices.

<sup>1</sup> These PROCEEDINGS, 30, 362-367(1944).

<sup>2</sup> Dickson, *Amer. Jour. Math.*, 57, 464(1935) proved this for the case  $n = 1$  and all the  $a$ 's are equal in (2), and our statement may be proved in the same way.

<sup>3</sup> These PROCEEDINGS, 31, 173-175(1945).

<sup>4</sup> *Amer. Jour. Math.*, 57, 391-424(1935); *Trans. Amer. Math. Soc.*, 38, 188-194(1935).

<sup>5</sup> *Ann. Math. II*, 18, 120(1917).

## PRIME NUMBER OF CONJUGATE OPERATORS IN A GROUP

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Suppose that every set of conjugate non-invariant operators of the group  $G$  involves the same prime number  $p$  of operators. The object of the present article is to determine some of the fundamental properties of the category of groups which satisfy this simple condition but are not otherwise restricted. The group of inner isomorphisms of  $G$  can be represented as an intransitive permutation group such that different letters are assigned to the different non-invariant operators of the group  $G$ . Each of the transitive constituents of this permutation group is of degree  $p$  since it is assumed that each of the non-invariant operators of  $G$  has exactly  $p$  conjugates under  $G$ . We shall first prove that each of the transitive constituents of the group of inner isomorphisms of  $G$  is a cyclic group of order  $p$  and of degree  $p$ .

Since the number of non-invariant conjugate operators of  $G$  is  $p$  the corresponding permutations in the group of inner isomorphisms of  $G$  cannot have more than  $p$  conjugates in its group of inner isomorphisms. Each of the transitive constituents of the group of inner isomorphisms of  $G$  must therefore be a transitive group of degree  $p$  which is either abelian or contains exactly  $p$  conjugates. As it must involve permutations of order  $p$  and none of these could have  $p$  conjugates since the number of the conjugates of an operator of order  $p$  in a transitive group of degrees  $p$  is always prime to  $p$ , it has been proved that all of the transitive constituents of the group of inner isomorphisms of  $G$  are regular groups of order  $p$ .

We have now established the following theorem: *If every non-invariant operator of a group has exactly  $p$  conjugates under the group its group of inner isomorphisms may be represented as an abelian intransitive permutation group all of whose transitive constituents are regular groups of order  $p$ . This group is of order  $p^m$  and of type  $I^m$ .* We can prove that  $m$  is always an even number in this theorem. It should be emphasized that the number of distinct operators in a set of conjugate operators of a group cannot be less than the number of operators in a set of conjugates of the corresponding operators in the group of inner isomorphisms. If the former number is greater than the latter it is a multiple of it. We shall now prove that  $m$  in the preceding italicized theorem is always even.

To prove this fact we may start with a subgroup of  $G$  which includes the central of  $G$  and whose order is  $p$  times the order of this central. This subgroup is abelian since the  $p$ th power of every operator of  $G$  appears in the central of  $G$ . It can be extended by an operator of  $G$  so as to obtain a sub-

group of  $G$  whose order is  $p^2$  times the order of the central of  $G$  and whose central is the central of  $G$ . It should be emphasized that this subgroup transforms its own operators in exactly the same manner as these operators are transformed under  $G$ . Hence it results that whenever the order of  $G$  exceeds  $p^2$  times the order of the central of  $G$  then the remaining operators of  $G$  must include operators which are commutative with every operator of this subgroup and have their  $p$ th power in the central of  $G$ . This results from the known theorem that if the operators of an invariant proper subgroup of a group are transformed under this subgroup in the same way as they are transformed under the group then the remaining operators of the group include at least one operator which is commutative with every operator of this invariant subgroup.

It results from the above that whenever the order of  $G$  exceeds  $p^2$  times the order of its central then  $G$  contains a subgroup whose order is  $p^4$  times the order of its central and that this invariant subgroup transforms its operators in exactly the same way as they are transformed under  $G$  if the order of  $G$  exceeds  $p^4$  times the order of the central of  $G$ . Since this process may be continued until  $G$  is reached whenever  $m$  is even and only then, it has been proved that  $m$  is an even number in the italicized theorem noted above. The proof is largely based on the fact that the invariant subgroups whose orders are even powers of  $p$  times the order of the central of  $G$  have this central for their own centrals and transform their own operators in exactly the same way as these operators are transformed under  $G$  itself.

The order of a commutator of  $G$  can clearly not exceed  $p$ . To prove that the order of the commutator subgroup of  $G$  can also not exceed  $p$  it may be noted that if the order of the commutator subgroup would be divisible by  $p^2$  we might suppose that  $t_1$  and  $t_2$  are two independent generators of the commutator subgroup of  $G$ . It may be assumed without loss of generality that the subgroup of  $G$  noted above whose order is  $p^2$  times the order of the central of  $G$  and which includes this central has the group generated by  $t_1$  as its commutator subgroup and includes  $s_1$  and  $s_2$  as its non-invariant operators which give rise to the commutator  $t_1$ . It may also be assumed that  $s_3$  and  $s_4$  are two other operators of  $G$  which are commutative with  $s_1$  and  $s_2$  but give rise to the commutator  $t_2$ . It would then follow that the product  $s_3s_4$  would have more than  $p$  conjugates under  $G$ . As this is impossible it has been proved that *the commutator subgroup of  $G$  is of order  $p$ .*

It results from Sylow's theorem that  $G$  contains only one Sylow subgroup whose order is a power of  $p$  since each of its operators is supposed to have no more than  $p$  conjugates under  $G$ . Since the largest subgroup of  $G$  whose order is prime to  $p$  appears in the central of  $G$  it is also invariant under  $G$ . It therefore results that  $G$  contains two invariant subgroups which have only the identity in common and hence it involves the direct



product of these two subgroups. Since the order of this direct product is equal to the order of  $G$  it has been proved that *if a group has the property that each of its non-invariant operators has a given prime number of conjugates under the group then it is the direct product of the Sylow subgroup whose order is a power of this prime number and an abelian subgroup whose order is prime to the given prime number.* The former of these two subgroups may be the identity.

Since the direct product of  $G$  and any abelian group whatever is again a group in which every non-invariant operator has the property that it has exactly  $p$  conjugates under the group it results that the two factor groups of  $G$  which are such that one is abelian and every non-invariant operator of the other has exactly  $p$  conjugates under it can usually be selected in various different ways. The main interest in  $G$  is its Sylow subgroup whose order is a power of  $p$ . Hence we may restrict our attention for the present to the study of  $G$  when it is a group whose order is a power of  $p$ . When  $p$  is odd all the operators of such a group, besides the identity, may be of order  $p$  but when  $p = 2$  some of the operators of such a group must be of order 4. The simplest example of such a group is then the octic group and the simplest example of  $G$  when  $p$  is any odd prime number is the non-abelian group of order  $p^3$  which involves no operator of order  $p^2$ .

In the octic group the number of the operators of order 2 is three more than the number of the operators of order 4. It may be of some interest to prove that this condition is also satisfied in the simplest group whose order is an arbitrary power of 2 and which satisfies the condition that each of its non-invariant operators has exactly two conjugates under the group. It was noted above that the order of such a group is an arbitrary odd power of 2 which is at least equal to the third power. This infinite system of simplest groups of this kind may be constructed by starting with the octic group and then constructing the direct product of this group and a group of order 2. This group of order 16 may then be extended by an operator of order 2 which is commutative with every operator of the given octic group and transforms each of the remaining operators into itself multiplied by the commutator of order 2 in the given octic group. We thus obtain the group of order 32 which belongs to the given infinite system and satisfies the condition that the number of its operators of order 2 is exactly three more than the number of its operators of order 4.

The system of odd prime power groups all of whose operators besides the identity are equal to this odd prime number includes one and only one abelian group for every power of this prime number but the number of such non-abelian groups for a given power of this prime number increases with the index of the power of this prime number. It follows from the preceding developments that this is always true even as regards the special class of these groups which satisfy the condition that each of the non-invariant

operators of the group has a number of conjugates under the group which is equal to this prime number  $p$ . The number of these groups of order  $p^m$  is  $(m - 1)/2$  when  $m$  is odd and  $(m - 2)/2$  when  $m$  is even since such a group is completely determined by the order of its central quotient group, and this order was proved above to be an even power of  $p$  whose index is less than  $m$  and greater than zero. In particular, there is one and only one such group of order  $p^3$  as well as of order  $p^4$  while there are two and only two such groups of each of the orders  $p^5$  and  $p^6$ , etc.

From the preceding developments we can formulate the following theorem: *If a group has the property that each of its non-invariant operators has the same prime number of conjugates under the group then its central quotient group has for its order an even power of this prime number, is abelian, and involves only operators besides the identity whose common order is this prime number.* While the orders of the operators in the central quotient group of this category of groups are therefore completely determined by the given condition the orders of the operators of the group are not limited thereby. It is evident that all the groups of this category are solvable, and that the category exhibits the fundamental nature of the concept of sets of conjugate operators in a group. It may be noted that whenever the number of conjugates in each set of conjugate non-invariant operators of a group is a prime number it may be equal to the same prime number in each case.



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## EXPERIMENTS ON SEXUAL ISOLATION IN *DROSOPHILA*. VI. ISOLATION BETWEEN *DROSOPHILA PSEUDOOBSCURA* AND *DROSOPHILA PERSIMILIS* AND THEIR HYBRIDS

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If males of either of the closely related species *Drosophila pseudoobscura* and *D. persimilis* have simultaneous access to females of both species, they inseminate a higher percentage of their own females. Particularly the males of *D. pseudoobscura* inseminate only a very small percentage of the available females of *D. persimilis*. Although the degree of preference can be altered slightly through conditioning, as is shown by the behavior of males previously associated with different kinds of females, there is little doubt that it is mainly controlled by genetic factors. It appeared therefore desirable to test the hybrids between the two species in regard to their position in the mating preference scale.<sup>1</sup>

*Material and Method.*—The same two stocks were used in this experiment, which have been described in an earlier paper in this series,<sup>1</sup> an orange-eyed strain of *Drosophila pseudoobscura* and a wild type strain of *D. persimilis*. The experimental procedure previously described was modified as follows: Males and females of the two species were segregated immediately after hatching and aged in food vials for a period of six to eight days. For each experiment ten or fewer males of one of the species were placed in a vial with food together with ten females of each of the two species or strains. The females were killed and examined for sperm, as described in an earlier paper,<sup>1</sup> after the number of hours necessary to insure the insemination of about fifty per cent of them had passed. The "experimental" and "aging" vials were kept in incubators at 24<sup>1</sup>/<sub>2</sub>°C.

*Hybrid Females.*—The female offspring of the cross of *D. pseudoobscura* with *D. persimilis* (formerly called *D. pseudoobscura B*) are fertile, while male hybrids are sterile.<sup>2</sup> Hybrid females of the two reciprocal crosses were tested with males and females of the two parental species. The results of these tests are summarized in table 1. It can be seen that males

of *D. persimilis* inseminate more hybrid females than females of their own species. Males of *pseudoobscura*, on the other hand, show a slight preference for their own females as compared to hybrid females. A definite maternal effect is apparent in both crosses: female hybrids whose mother is conspecific with the tested males are inseminated more frequently than females of the reciprocal cross (table 1).

TABLE 1

NUMBER OF FEMALES DISSECTED (*n*) AND PER CENT CARRYING SPERM (%) IN WHICH MALES HAD THE CHOICE BETWEEN FEMALES OF THEIR OWN SPECIES AND ALIEN FEMALES

MALE AND HOMO- GAMIC FEMALE	ALIEN FEMALE (HETEROGAMIC)	HOMOGAMIC " %	HETEROGAMIC " %	$\chi^2$	ISOLATION INDEX
<i>persimilis</i>	<i>persimilis</i> × <i>pseudoobscura</i> <sup>H</sup>	68 38.2	68 73.5	17.2	-0.32
<i>persimilis</i>	<i>pseudoobscura</i> × <i>persimilis</i> <sup>H</sup>	142 33.8	143 45.5	4.04	-0.15
<i>pseudoobscura</i>	<i>pseudoobscura</i> × <i>persimilis</i> <sup>H</sup>	142 69.0	145 54.5	5.76	+0.11
<i>pseudoobscura</i>	<i>persimilis</i> × <i>pseudoobscura</i> <sup>H</sup>	80 67.5	84 27.4	29.7	+0.42
<i>persimilis</i>	<i>pseudoobscura</i> <sup>C</sup>	107 72.0	97 41.3	19.5	+0.27
<i>pseudoobscura</i>	<i>persimilis</i> <sup>C</sup>	202 77.3	205 6.8	207.7	+0.84

<sup>H</sup> Hybrid females.

<sup>C</sup> Control females.

In the first two experiments *persimilis* males are given the choice between their own (homogamic) and hybrid females. They inseminate more hybrid than own females. In the next two experiments *pseudoobscura* males are given a choice of their own and hybrid females. They show a slight preference for their own females. A maternal effect is apparent in both cases. In the two control experiments males are given a choice of females of their own and of the other species.

*Discussion.*—Direct observations of the mating behavior of *Drosophila* indicate that the "ratio of preference" in the main is controlled by three factors.<sup>3</sup> Species recognition or attraction is one of them, physical compatibility of the genitalia is the second and the degree of activity is the third. The more active a fly (of either sex), the more readily it will participate in a copulation. The factor of activity is particularly important in the case of hybrid females in which hybrid vigor may compensate for an adverse influence of the two other factors. In control tests (table 1, see also earlier paper<sup>1</sup>) it was shown that males of *D. persimilis* have a much lower ratio of preference for their own females than males of *D. pseudoobscura*. Males of *persimilis* inseminate about twice as many of their own as of alien females if equal numbers of both are available. Males of *pseudoobscura* inseminate more than ten times as many of their own females as of *persimilis*. This might mean that factors one, two or both are less important for the males of *D. persimilis* than for those of *pseudo-*

*obscura*. The results of the experiments with hybrids are consistent with this hypothesis (table 1). Males of *D. persimilis* inseminate a higher percentage of the hybrid than of their own females. The isolation index<sup>1</sup> is negative in both crosses ( $-0.15$ ,  $-0.32$ ). The greater activity of the hybrid females is apparently more than sufficient with males of *D. persimilis* to compensate for their genetic inferiority in regard to factors one and two. The greater activity of the hybrid females is not quite sufficient in tests with the males of *D. pseudoobscura* to overcome the adverse influence of factors one and two. The isolation index remains positive ( $+0.11$ ,  $+0.42$ ). Still, the discrimination of the *pseudoobscura* males against hybrid females is much slighter than against *persimilis* females. At best (with *persimilis* ♀ × *pseudoobscura* ♂ hybrids), only twice as many of their own females are inseminated as against ten times as many in the control experiment.

The relative desirability of the hybrid females is a puzzling fact, considering the wide overlap of the two species in nature. There would seem to be an apparent opportunity for a good deal of introgressive hybridization. The factors that keep this potential danger in check need further investigation.

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<sup>2</sup> Lancefield, D. E., *Zeits. ind. Abs. Vererbungsl.*, 52, 287-317 (1929).

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## INHERITED DIFFERENCES IN SENSITIVITY TO RADIATION IN *ESCHERICHIA COLI*\*

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The study of spontaneous and radiation-induced mutations is at present our best approach to the investigation of genetic mechanisms in bacteria. Mutations involving resistance to destructive agents (bacteriophage,<sup>1</sup> penicillin<sup>2</sup>) are especially suitable for genetic analysis, since resistant mutants can easily be detected in bacterial cultures. This preliminary report concerns a mutation in *Escherichia coli* leading to resistance to both ultraviolet radiation and x-rays, which was detected by exposing samples from normal cultures to high doses of radiation.

Most investigators of the effects of ultraviolet radiation on bacteria have considered the population within a strain to be fundamentally uniform in sensitivity. Most of the differences found seem to depend upon transient

physiological factors (age of cells, conditions of culture). Rentschler, Nagy and Mouromoff,<sup>3</sup> however, reported genetic differences in sensitivity to ultraviolet in *E. coli*, and Hollaender<sup>4</sup> noted that, in a population of *E. coli*, one bacterium in a million could survive irradiation with very high doses.

*Material and Methods.*—Strain *B* of *Escherichia coli* was used throughout these experiments. A stock slant was established from a single-colony isolation at the start, and was subcultured every two months. Cultures inoculated with samples from the same slant were used for all comparable experiments.

Difco nutrient broth and nutrient agar were the media used.

TABLE 1

SENSITIVITY TO ULTRAVIOLET OF BACTERIA SURVIVING IRRADIATION WITH A HIGH DOSE OF ULTRAVIOLET

Origin of control cultures: single colonies from a non-irradiated plate seeded with bacteria from strain *B*. Origin of experimental cultures: single-colony survivors from plates seeded with bacteria from strain *B*, and irradiated with a dose of 1000 ergs/mm.<sup>2</sup>.

CULTURE	TREATMENT: 90 ERGS/MM. <sup>2</sup>			TREATMENT: 550 ERGS/MM. <sup>2</sup>		
	CELLS PER SAMPLE	SURVIVORS NUMBER	PER CENT	CELLS PER SAMPLE	SURVIVORS NUMBER	PER CENT
Control—1	520	18	3.5	1040	4	0.4
Control—2	481	26	5.4	962	5	0.5
Control—3	456	13	2.9	912	8	0.9
Control—4	509	30	5.9	1018	2	0.2
Experimental—1	455	422	92.9	890	336	37.7
Experimental—2	520	507	97.5	1040	416	40.0
Experimental—3	490	482	98.3	980	424	43.2
Experimental—4	512	473	92.3	1024	391	38.1

The source of ultraviolet radiation was a General Electric low-pressure mercury-vapor lamp, emitting unfiltered radiation primarily of wavelength 2537 Å. Doses are expressed in ergs per mm.<sup>2</sup>, but these values are approximate, since an indirect biological method of calibration was used.

Bacteria to be irradiated with ultraviolet were taken from 24-hour broth cultures and diluted quantitatively in broth. Measured samples were spread evenly on the surface of nutrient-agar Petri dishes with a sterile glass rod. The plates were then exposed to the radiation, and colony counts were made after 24 hours of incubation. Survival was measured by comparison with non-irradiated control plates.

Irradiation with x-rays was conducted at Memorial Hospital in New York City, through the courtesy of Mr. L. D. Marinelli, and with the assistance of Miss E. Focht. The source emitted unfiltered rays of 180 kv., with an intensity of 2050 roentgens per minute.

Bacteria to be irradiated with x-rays were taken from undiluted 24-hour aerated broth cultures, and were exposed in small, thin-walled glass tubes.

Measured dilutions were made after irradiation, and were plated out. Colony counts were made after incubation, and compared with non-irradiated controls.

Assays to determine the titre of liquid cultures were made by plating measured dilutions and making colony counts.

*Experimental Results.—Derivation of the Resistant Strain:* A sample of about  $5 \times 10^4$  bacteria from a culture of *B* was irradiated with a dose of 1000 ergs per mm.<sup>2</sup>. After 24 hours of incubation, 4 colonies had developed, indicating that only 4 bacteria had survived the irradiation. These colonies were inoculated separately into broth, and samples from the resulting cultures were irradiated to determine their sensitivity to 2 test doses of ultraviolet. Four control cultures, started from single colonies on a non-irradiated plate, were irradiated with the same doses, and the survival of the 2 sets of cultures was compared. Table 1 gives the results, which indicate that all 4 survivors were characterized by markedly greater resistance to ultraviolet than the normal strain.

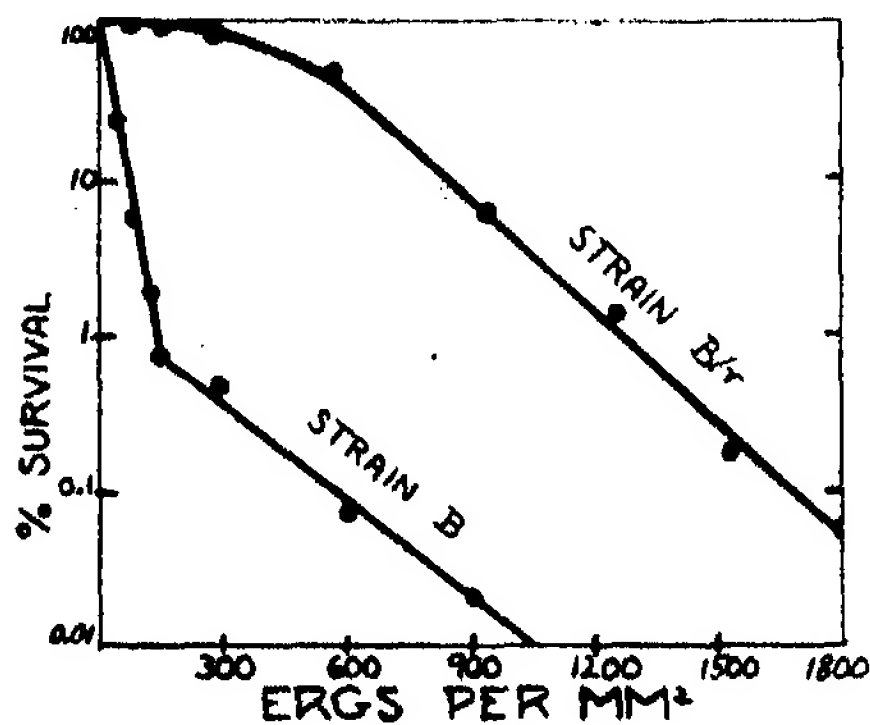


Figure 1

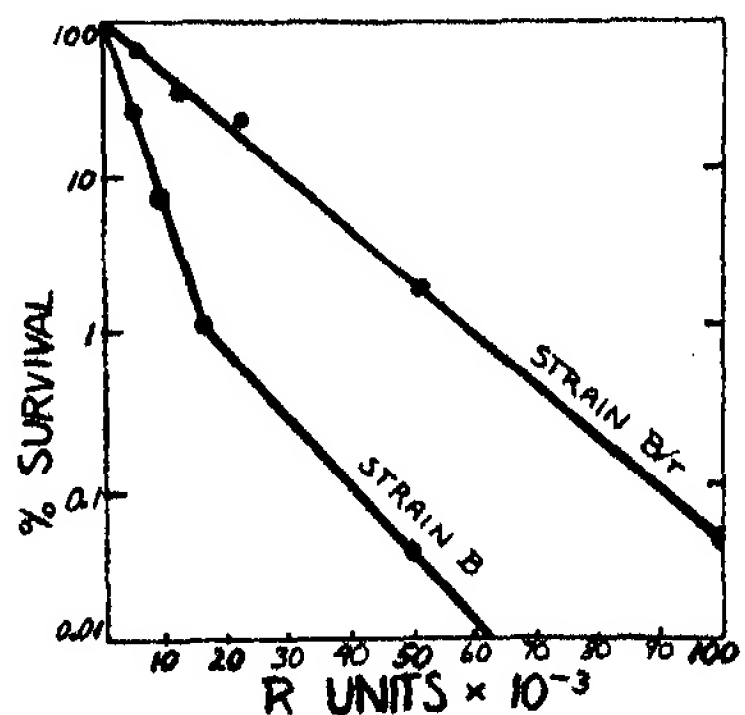


Figure 2

Figure 1. Ultraviolet Survival Curves of Strains *B* and *B/r*.Figure 2. X-Ray Survival Curves of Strains *B* and *B/r*.

One of these 4 resistant cultures (No. 1) was established on an agar slant to serve as a stock-resistant strain for further study. This strain will be referred to as strain *B/r* (*B* resistant to radiation).

*Properties of the Resistant Strain:* (1) *Stability of Strain B/r.*—The resistant strain *B/r* has been carried through over 50 subcultures in broth, and for a period of 18 months on agar, with no change in ultraviolet sensitivity or in other observed properties. Ultraviolet resistance, therefore, may be considered a stable, heritable character.

(2) *Sensitivity of Strain B/r to Ultraviolet.*—The curves of survival of strains *B* and *B/r* as a function of ultraviolet dose are given in figure 1. Single-colony survivors were isolated from various points along the curve



of strain *B*, and were tested for sensitivity to ultraviolet. Only the two levels of sensitivity represented by strains *B* and *B/r* were observed. No intermediate resistance, and no resistance greater than that of *B/r* were detected.

(3) *Sensitivity of Strain B/r to X-rays.*—Figure 2 gives survival curves of strains *B* and *B/r* with x-rays. It is evident that strain *B/r* is relatively resistant to x-rays, as well as to ultraviolet radiation.

The survival curves of the normal strain with both ultraviolet and x-rays show a change in slope at about 1% survival. The change in killing rate at this point can be partially, but not entirely, explained by the presence of resistant bacteria in the normal sample.

(4) *Growth Rate of Strain B/r.*—Growth curves of strains *B* and *B/r* in broth at 37°C. were compared. The lag phase of *B/r* was found to be about 25% shorter than that of the normal strain. The generation time was the same for the two strains (19 minutes).

*A Technique for the Quantitative Detection of Radiation-Resistant Bacteria in Samples from Normal Cultures:* Before the genetic basis of resistance to radiation could be determined, it was necessary to overcome a methodological obstacle. The difficulty was based on the fact that resistance to radiation is relative, rather than absolute.

If spontaneous mutation is wholly or partially responsible for the change in sensitivity, a normal culture must contain, prior to irradiation, a certain proportion of resistant cells. A glance at the survival curves (Figs. 1 and 2) will show that a dose high enough to eliminate all or most of the sensitive bacteria will also eliminate most of the resistant cells. Treatment with a high dose of ultraviolet, therefore, will permit the recovery of only a small fraction of the resistant bacteria originally present in the sample.

Since an accurate determination of the number of resistants present in various samples is necessary for any quantitative study of the origin of the resistant bacteria, a method was developed whereby all the resistant bacteria in a normal sample could be detected.

The first clue to this technique was obtained by observing ultraviolet-irradiated bacteria of strains *B* and *B/r* under the microscope. It is well known that irradiated bacteria, within a certain range of doses, grow in length for several hours before dividing, forming snake-like filaments sometimes hundreds of times their normal length.

Samples of *B* and *B/r* were irradiated with a dose of 50 ergs per mm.<sup>2</sup>, which permits 100% survival of resistant bacteria and reduces the number of sensitive bacteria to about 10%. The irradiated plates were incubated, and the bacteria were examined at intervals under the microscope.

After 3 hours, the irradiated cells of strain *B* were extremely elongated and still undivided. Division of the resistant cells, however, was not inhibited by this dose, and after 3 hours each originally present resistant



bacterium had given rise to a microcolony of about 100 cells of normal length.

The plates were given a second irradiation of 700 ergs per mm.<sup>2</sup>, 3 hours after the first. On the one hand, this treatment should reduce each resistant microcolony to about 10 cells. If at least one cell in each resistant microcolony survives this second treatment, a visible colony for every originally present resistant bacterium will form. On the other hand, the second irradiation should eliminate all the sensitive survivors of the first treatment, if the number of bacteria in the original sample is not more than  $2 \times 10^4$ , and if the elongated cells resulting from the first irradiation behave like single bacteria rather than like chains of bacteria in their sensitivity to ultraviolet. Lea, Haines and Coulson<sup>6</sup> found that this was true of long forms produced by gamma rays.

TABLE 2

RELIABILITY OF DOUBLE-IRRADIATION TECHNIQUE FOR SELECTIVE RECOVERY OF RADIATION-RESISTANT BACTERIA IN MIXTURE WITH SENSITIVE BACTERIA

Double-irradiation technique: first dose of 50 ergs/mm.<sup>2</sup> followed by 3 hours of incubation and second dose of 700 ergs/mm.<sup>2</sup>.

CULTURE	SAMPLE	INITIAL NUMBER OF CELLS PER SAMPLE		COLONY COUNT AFTER TREATMENT AND INCUBATION	
		<i>B</i>	<i>B/r</i>	SENSITIVE	RESISTANT
Normal ( <i>B</i> )	1			0	1
	2			0	0
	3			0	0
	4			0	1
		Av. 11,200	...	...	...
Resistant ( <i>B/r</i> )	1			0	114
	2			0	91
	3			0	120
	4			0	130
		...	Av. 111 $\pm$ 9.0	...	Av. 113.7 $\pm$ 16.5
Mixture ( <i>B</i> + <i>B/r</i> )	1			0	94
	2			0	112
	3			0	106
	4			0	96
		Av. 9,400	Av. 106 $\pm$ 8.5	...	Av. 102.0 $\pm$ 8.5

Table 2 gives results of an experiment to determine the validity of this technique. Samples of *B*, *B/r* and of a mixture of the two strains were given this double-irradiation treatment, and the results, which were repeatedly confirmed, indicate that it is effective in permitting selective survival of resistant bacteria.

The technique can be modified to determine the number of resistant bacteria in samples as large as  $10^7$ , by extending the time of incubation between irradiations up to 5 hours, and increasing the second dose up to 1800 ergs per mm.<sup>2</sup>. When large samples are used, however, sensitive

bacteria occasionally survive, probably because of screening due to the dense network of elongated cells which develops after the first irradiation. When samples of about  $10^6$  or more bacteria are used, it is necessary, therefore, to determine the sensitivity of each surviving colony. This can be done rapidly by suspending bacteria from the colony to be tested in a drop of broth, spreading the suspension on agar and irradiating with a dose of 50 ergs per mm.<sup>2</sup>. After 3 hours of incubation, the bacteria are examined under the microscope. If the colony consisted of sensitive bacteria, the microscope will reveal thread-like elongated cells. If the colony consisted of resistant bacteria, microcolonies of about 100 cells of normal length will be observed.

*Origin of the Change from Sensitivity to Resistance:* It is important to establish the mode of origin of hereditary variations in bacteria. In the case of radiation-resistance the problem is of particular interest, since ultraviolet radiation and x-rays are known to be effective in inducing mutations.

The possible modes of origin may be considered as follows: (1) The change to increased resistance is induced by the radiation in a certain number of cells in an initially homogeneous population; or (2) the change is a spontaneously occurring mutation, and prior to the treatment the culture contains a certain number of resistant mutants. In this case (a) the radiation acts merely as a selective agent, or (b) the radiation acts as a selective agent, but also acts as an inducing agent, increasing the rate of mutation to resistance.

The method used to test these hypotheses was that developed by Luria and Delbrück<sup>1</sup> and applied by them and by Demerec<sup>2</sup> to the study of bacterial mutations. The method involves the following considerations:

If the change is entirely induced by the radiation, the number of resistant bacteria obtained from a sample will depend upon the probability that an induced change will occur in any bacterium. This probability should be the same for all bacteria under similar physiological conditions. Therefore, the number of resistants in samples from a series of similar, independent cultures should show fluctuations no greater than those shown by the number of resistants in a series of samples from a single culture. These fluctuations should be due only to sampling error, and in both cases the distribution of the number of resistants should constitute a Poisson series, with the variance approximately equal to the mean.

If the change is a spontaneous mutation, the number of resistants obtained from a given sample depends upon: (1) the probability that any bacterium will mutate during its lifetime, and (2) the time of occurrence of mutations during the growth of the culture, since all bacteria descended from mutated cells will be resistant. In this case, the number of resistants in samples from a series of similar, independent cultures should show large

fluctuations (see Luria and Delbrück<sup>1</sup>), and the variance should be significantly higher than the mean.

Table 3 gives results of experiments to determine the number of resistant bacteria in samples from a series of similar, independent cultures and in samples from a single culture. Every culture was started with an inoculum of about 20 cells from the normal strain, in a volume of 1 ml. of broth. The final titre of the cultures in any series differed by not more than 12%.

TABLE 3  
NUMBER OF RESISTANT BACTERIA IN SAMPLES FROM INDEPENDENT CULTURES, AND IN SAMPLES FROM SINGLE CULTURES

Number of resistant bacteria determined by double-irradiation technique: first dose of 50 ergs/mm.<sup>2</sup> followed by 5 hours of incubation, and second dose of 1500 ergs/mm.<sup>2</sup>.

SAMPLES FROM INDEPENDENT CULTURES				SAMPLES FROM SINGLE CULTURES			
RXPT. NO.	1	2	3	1	2	3	
AV. NO. CELLS PER SAMPLE	$1 \times 10^6$	$1.1 \times 10^6$	$9.5 \times 10^5$	$9.7 \times 10^5$	$1 \times 10^6$	$1.2 \times 10^6$	
CULTURE NO.				SAMPLE NO.			
1	0	4	5	1	8	0	13
2	12	8	13	2	10	1	9
3	8	15	61	3	5	3	11
4	8	12	10	4	9	1	14
5	19	0	1	5	7	0	9
6	98	14	12	6	9	0	8
7	7	1	2	7	15	2	12
8	5	76	8	8	8	1	13
9	14	11	0	9	6	4	7
10	24	42	13	10	16	1	15
11	..	..	9	11	..	..	8
12	..	..	7	12	..	..	8
13	..	..	18	13	..	..	14
14	..	..	0	14	..	..	9
15	..	..	11	15	..	..	13
16	..	..	8	16	..	..	6
17	..	..	116	17	..	..	11
18	..	..	12	18	..	..	7
19	..	..	10	19	..	..	10
20	..	..	6	20	..	..	18
Average	19.5	18.3	18.1	..	9.3	1.3	10.8
Variance	764.7	574.7	1509.8	..	12.9	1.8	6.9
$\chi^2$	373.3	279.6	844.0	..	12.5	11.6	12.2
P	..	..	..	..	0.1-0.2	0.2-0.3	0.8-0.9

In all three experiments, the number of resistants in samples from a single culture shows fluctuations satisfactorily accounted for by sampling errors. This indicates that the method of plating and irradiating does not introduce fluctuations beyond those expected on the basis of sampling.

The number of resistants in samples from a series of independent cultures,

in all three experiments, shows fluctuations much greater than can be accounted for by sampling errors. The variance is significantly higher than the mean in every case, and the fluctuations are of the type to be expected according to the hypothesis of spontaneous mutation.

The possibility remains that, while radiation-resistance occurs as a spontaneous mutation, the treatment used to detect these mutants induces the change in an additional number of sensitive bacteria. Indeed, such an effect should be expected on the basis of the known power of ultraviolet to induce mutations. Under the conditions of the experiment, however, it is not likely that induced mutations could be detected. The double-irradiation technique permits the survival only of bacteria that are resistant at the time of the treatment—no sensitive cell has a chance to survive. Therefore, sensitive bacteria in which resistance is induced during the treatment will survive only if they become phenotypically resistant *immediately*. The following evidence suggests that induced mutations, if they occur, are not detected by the double-irradiation technique. The doses used in this treatment, depending upon the initial number of bacteria in the sample, vary from 700 to 1800 ergs per mm.<sup>2</sup> If the number of mutants in several samples from the same culture is determined, using doses throughout this range, the proportion of mutants in the various samples is found to be the same. If these mutants arise by induction, their number should be proportional to the dose.

*The Mutation Rate:* It is possible to estimate the rate of bacterial mutations from experiments of the type described above by solving the following equation:  $r = aN_i \ln (CaN_i)$  (for derivation see Luria and Delbrück<sup>1</sup>), where  $r$  is the experimental average of the number of mutants in a series of similar cultures,  $N_i$  is the number of bacteria at the time of observation,  $C$  is the number of cultures and  $a$  is the mutation rate.

Luria and Delbrück have plotted a series of curves relating the observed values of  $r$  to  $aN_i$  for various values of  $C$ . The estimated mutation rate for radiation-resistance, obtained from these curves, is about  $10^{-5}$  mutations per bacterium per generation.

*Discussion.*—The evidence presented indicates that the heightened resistance to radiation exhibited by bacteria of strain  $B/r$  is the expression of a mutation which occurs spontaneously in cultures of strain  $B$ . No critical evidence is available which precludes the possibility that the observed change in sensitivity may be produced by different mutations. However, unrelated resistant strains, each isolated from a different single-cell culture of strain  $B$ , exhibit similar sensitivity to ultraviolet and to x-rays, similar growth rates and growth requirements, similar colony characteristics and similar patterns of individual cell growth and division after irradiation. If different mutations give rise to radiation-resistance, their effects must be identical in so far as these properties are concerned.

The fact that *B/r* is resistant to both ultraviolet and x-rays suggests that the mutation affects a process which is involved in the lethal action of both types of radiation, unless it is assumed that the mutation involves more than one fundamental change.

The mechanism of the lethal action of radiations on bacteria is so poorly understood that it is difficult to speculate about the changes that might be responsible for the heightened resistance of the mutant *B/r*. The survival curves (Figs. 1 and 2), if interpreted on the basis of the hit theory of radiation effects, suggest a possible difference between sensitive and resistant bacteria. The ultraviolet and x-ray survival curves of strain *B* are both exponential "one-hit-to-kill" curves. The ultraviolet survival curve of strain *B/r* may be interpreted as a multiple-hit curve, and suggests that this strain differs from the normal strain in possessing multiple centers of lethal action. The exponential x-ray curve of *B/r* seems to contradict this idea, but could be explained by the assumption that a single x-ray "hit" may consist of several ionizations, and may produce more than one change, a possibility stressed recently by Lea and Catcheside.<sup>6</sup>

Another clue to the mechanism of resistance is obtained from the microscopic observation of ultraviolet-irradiated bacteria of strains *B* and *B/r*. The radiation exerts a strong inhibitory effect on cell division in sensitive bacteria, resulting in the production of long forms. The division mechanism of resistant bacteria is not appreciably inhibited by the same dose that delays division in strain *B* for several hours, indicating that the resistance to radiation is a specific resistance of the division mechanism. The harder division mechanism of strain *B/r* may be seen as the cause of the shorter lag phase of that strain, which may or may not be related to its radiation-resistance.

Lea, Haines and Coulson<sup>5</sup> have considered the lethal action and the inhibition of division as independent effects of gamma rays. Bacteria of strain *B/r* differ from sensitive bacteria in degree of susceptibility to both the lethal action and the division-inhibiting action of ultraviolet. Again, unless two basic differences between the two strains are assumed, it seems likely that the power to inhibit division and the lethal effect, at least of ultraviolet, are directly related.

**Summary.**—(1) Strain *B* of *Escherichia coli* yields variants which are characterized by resistance to both ultraviolet radiation and x-rays, and which can be detected by the selective action of these radiations.

(2) Resistance to ultraviolet radiation and x-rays is a stable, heritable character.

(3) A technique is described whereby the number of resistant bacteria in samples from normal cultures can be determined accurately.

(4) The change from sensitivity to resistance is a spontaneous muta-



tion occurring in normal cultures at a rate of about  $1 \times 10^{-5}$  mutations per bacterium per generation.

(5) Doses of ultraviolet that inhibit cell division in sensitive bacteria for several hours, resulting in the production of elongated cells, do not appreciably inhibit division in resistant bacteria.

(6) Curves of survival of sensitive and resistant bacteria as a function of ultraviolet and x-ray doses are compared.

(7) Interpretations of certain features of the results are discussed.

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## A NEW GENE THEORY AND AN EXPLANATION OF THE PHENOMENON OF DOMINANCE TO MENDELIAN SEGREGATION OF THE CYTOGENE

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The chromosomes have often been called the "bearers" of the hereditary factors and the terms "gene" and "locus" have been considered synonymous. This terminology was prophetic of the real nature of the hereditary mechanism. The experiments presented herewith show that the locus, which I propose to call the *chromogene*, is simply a place of attachment for the cytogene. This view corroborates the principle of the duality of the gene originally announced by Sonneborn,<sup>3</sup> with this significant difference: the cytogene<sup>1, 2, 4</sup> is an entity capable of self-duplication in the cytoplasm. Sonneborn's kappa substance only multiplies in the macronucleus; it does not multiply in the cytoplasm. The cytogene may be transmitted from parent to offspring either by being held at the dominant chromogene in the absence of its specific substrate, or by continued multiplication in the cytoplasm of cells containing the recessive chromogene if the specific substrate is present. A cell containing the dominant (fermenting) gene is capable of fermenting a specific carbohydrate, thus indicating that it is



capable of releasing the cytogene. The cytogene may be transferred through the cytoplasm to a cell which contains the recessive (normally non-fermenting) locus. These contaminated (by the cytogene) recessive cells are able to continue to ferment a specific carbohydrate for variable periods of time, in some cases as long as they are kept in contact with the substrate, but when the substrate is permanently removed they may lose their fermentative ability. In some recent experiments we mated a clone carrying the dominant (fermenting) allele to a clone carrying the recessive (non-fermenting) allele. Some of the recessive haploid clones obtained from the hybrid were contaminated by the cytogene. We may then speak of (1) dominant, (2) contaminated and (3) recessive alleles. When a contaminated by recessive hybrid underwent reduction, regular segregation of fermenter to non-fermenter occurred in most asci. Therefore, a cytoplasmic character exhibited Mendelian segregation. Further details and protocols will be reported elsewhere.

This phenomenon indicates the true nature of the gene: it is a duality consisting of two self-duplicating entities: (1) the chromogene, a chromosomal unit to which cytogenes are attached; and (2) the cytogene, an entity which multiplies in the cytoplasm independently of the chromogene. Recessive and dominant chromogenes differ in their affinities for the cytogene. The dominant (fermenting) chromogene has a greater affinity for the cytogene than its recessive allele, and when segregation of a dominant/recessive hybrid occurs, the dominant chromogene collects practically all the cytogenes and transmits them to its progeny.

In a contaminated recessive (carrying the cytogene) the recessive allele has some affinity for the cytogene and so carries a few of the enzyme molecules on its surface. In a contaminated/recessive hybrid, these cytogenes form the basis for the attraction of practically all the cytogenes (similar to centers of crystallization) which gives the contaminated locus an advantage over the recessive in competition for the free cytogenes. In this competition the recessive allele fails to collect any cytogenes and when the contaminated/recessive hybrid undergoes reduction, the contaminated allele receives all the cytogenes. However, the affinity of the recessive allele is small and it occasionally fails to maintain contact with the cytogene. In this case the cell permanently loses its capacity to ferment the carbohydrate. The dominant chromogene has a much greater affinity and practically never loses all its cytogenes. According to the theory, the chromogene is self-duplicating at each chromosome division but it is not a "generator" of anything except itself.

The fermentation of a carbohydrate is often effected by an adaptive enzyme and a certain specific time period intervenes between exposure of the cell to the carbohydrate and the production of a measurable amount of  $\text{CO}_2$ . If one supposes that the specific carbohydrate like the chromo-

gene has an affinity for the cytogene, this lag period is the time required for transfer of the cytogene from the chromogene to the cytoplasm and the development of a measurable amount of  $\text{CO}_2$ . According to this view, the chromogenes are occupied by cytogenes at those times when substrate is absent from the cell. When the substrate appears, the diffusion gradient toward it robs the chromogene of most of its cytogenes. When the substrate has been transformed, the cytogenes return to the locus. If the substrate is one rarely encountered, the stored cytogenes may be called forth only rarely. The cytogenes diffuse from the chromogene into the cytoplasm where they transform a specific substrate and duplicate themselves at the same time. After all the substrate has been transformed, a few molecules return to the chromogene and the excess of cytogenes is converted into other similar enzymes. Plasmagenes or viruses are modified cytogenes which can be transmitted without recourse to a chromosome locus.

Simple "loss" mutations may be the result of either (1) transforming the chromogene into a site which no longer has any affinity for the cytogene, or (2) complete destruction or loss of the cytogene. Some hypomorphic mutations may be changes in the locus which reduce the affinity of the chromogene for the cytogene. Other mutations may be alterations in the structure of the cytogene or simultaneous alteration in chromogene and cytogene.

<sup>1</sup> Lindegren, C. C., *Ann. Mo. Bot. Gard.*, **32**, 107-123 (1945).

<sup>2</sup> Lindegren, C. C., Spiegelman, S., and Lindegren, G., these PROCEEDINGS, **30**, 346-352 (1944).

<sup>3</sup> Sonneborn, T. M., *Ann. Mo. Bot. Gard.*, **32**, 213-221 (1945).

<sup>4</sup> Spiegelman, S., Lindegren, C. C., and Lindegren, G., these PROCEEDINGS, **31**, 95-102 (1945).

## AN EXTENSION OF SCHUSTER'S INTEGRAL

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1. Schuster's integral, which occurs in the theory of total reflection of light,<sup>1</sup> is

$$\int_0^\infty (C^2 + S^2) dx = (\pi/8)^{1/2}, \quad C = \int_0^\infty \cos t^2 dt, \quad S = \int_0^\infty \sin t^2 dt$$

where the notation is that used by Nielsen<sup>2</sup> and Hardy.<sup>3</sup> An extension is obtained by modifying the analysis of Ingham.<sup>4</sup> If

$$C(x) = \int_0^\infty \cos(t^n) dt, \quad S(x) = \int_0^\infty \sin(t^n) dt \quad (n > 1)$$

the extension of Ingham's lemma is

$$\begin{aligned} C(x) &= (1/n) \int_x^\infty (dt/t)(d/dt)(\sin t^n) \\ &= -(1/nx) \sin(x^n) + (1/n) \int_x^\infty \sin(t^n) dt/t^2 = O(1/x) \end{aligned}$$

when  $x$  is large and positive. It is easily seen also that  $S(x) = O(1/x)$  and so if

$$I = \int_0^\infty C(x)C(ax)x^{n-2}dx, \quad J = \int_0^\infty S(x)S(ax)x^{n-2}dx \quad (a > 0)$$

integration by parts gives

$$\begin{aligned} n(n-1)I &= n \int_0^\infty x^{n-1}dx [\cos(x^n)C(ax) + a \cos(a^n x^n)C(x)] \\ &= a \int_0^\infty \sin(x^n) \cos(a^n x^n)dx + a^{1-n} \int_0^\infty \sin(a^n x^n) \cos(x^n)dx \\ &= \frac{1}{2}a^{1-n}S(0)(s^m - d^m), \quad S = 1 + a^n, \quad d = |1 - a^n|, \\ &\quad m = 1 - 1/n \end{aligned}$$

$$\begin{aligned} n(n-1)J &= n \int_0^\infty x^{n-1}dx [\sin(x^n)S(ax) + a \sin(a^n x^n)S(x)] \\ &= (1 + a^{1-n})S(0) - a \int_0^\infty \cos(x^n) \sin(a^n x^n)dx - a^{1-n} \int_0^\infty \cos(a^n x^n) \sin(x^n)dx \\ &= \frac{1}{2}a^{1-n}S(0)[2(1 + a^{n-1}) - s^m - d^m], \quad S(0) = \frac{1}{n\Gamma\left(\frac{1}{n}\right)} \times \\ &\quad \sin(\pi/2n). \end{aligned}$$

In particular, when  $n = 2$

$$\begin{aligned} I &= (1/4a)[(1 + a^2)^{1/2} - |1 - a^2|^{1/2}]S(0) \\ J &= (1/4a)[2(1 + a) - (1 + a^2)^{1/2} - |1 - a^2|^{1/2}]S(0) \\ I + J &= (1/2a)S(0)[1 + a - |1 - a^2|^{1/2}] \\ J - I &= (1/2a)S(0)[1 + a - (1 + a^2)^{1/2}]. \end{aligned}$$

In these equations  $C(0) = S(0) = (\pi/8)^{1/2}$  and so when  $a = 1$  the third equation gives Schuster's relation. We also have

$$\int_0^\infty C(x)S(ax)dx = (1/4a)C(0)[2 \pm |a^2 - 1|^{1/2} - (a^2 + 1)^{1/2}]$$

where the upper or lower sign is taken according as  $a$  is greater or less than one. Similarly,

$$\int_0^\infty S(x)C(ax)dx = (1/4a)C(0)[2a \mp |a^2 - 1|^{1/2} - (a^2 + 1)^{1/2}].$$

Returning to the more general case in which  $n$  does not have the special value 2 we note that if

$$K = \int_0^\infty C(x)S(ax)x^{n-2}dx \quad (n > 1)$$

$$n(n-1)K = \frac{1}{2}a^{1-n}C(0)[2 \pm d^m - s^m], \quad C(0) = \frac{1}{n}\Gamma\left(\frac{1}{n}\right) \cos(\pi/2n).$$

2. A number of other integrals may be derived from the values of  $I$ ,  $J$ ,  $K$  with the aid of the relations

$$\begin{aligned}\int_0^\infty a^{s-1} C(ax) da &= \frac{x^{-s}}{nz} \Gamma\left(\frac{1+z}{n}\right) \cos\left[\frac{\pi(1+z)}{2n}\right] \\ \int_0^\infty a^{s-1} S(ax) da &= \frac{x^{-s}}{nz} \Gamma\left(\frac{1+z}{n}\right) \sin\left[\frac{\pi(1+z)}{2n}\right]\end{aligned}\quad (z > -1).$$

Thus from  $I$  we obtain the relation

$$\int_0^\infty t^{s-1} [(1+t)^p - |1-t|^p] dt = -\frac{p}{z\Gamma(2-p)} \sec\left(\frac{1}{2}p\pi\right) \quad (-1 < z < 1-p).$$

3. We next write for brevity

$$\int_x^\infty e^{-t^n} dt = E(x) \quad (n > 1).$$

Then, if

$$L = \int_0^\infty x^{n-2} E(x) E(ax) dx$$

we find on integration by parts that

$$n(n-1)L = a^{1-n} [1 + a^{n-1} - (1 + a^n)^{1-1/n}] E(0).$$

The transformation  $u = t^n$  indicates that  $E(0) = \Gamma(1 + 1/n)$

$$E(x) = (1/n) \Gamma(1/n, x^n)$$

consequently, the equation may be written in the form

$$\int_0^\infty \Gamma(m, u) \Gamma(m, xu) du / u^m = \Gamma(m-1) [(1 + 1/x)^{m-1} - 1 - x^{m-1}] \quad (0 < m < 1).$$

In the particular case in which  $n = 2$

$$\int_0^\infty E(x) E(ax) dx = (1/a) [1 + a - (1 + a^2)^{1/2}] E(0)$$

and  $E(0) = \frac{1}{2}\pi$ .

<sup>1</sup> Schuster, Sir Arthur, *Proc. Roy. Soc. London*, 107A, 15-30 (1925).

<sup>2</sup> Nielsen, N., *Theorie des Integrallogarithmus und verwandter Transzendenten*, Leipzig, 1906.

<sup>3</sup> Hardy, G. H., *Proc. London Math. Soc.*, 24 (2), Records for Feb. 12 (1925). In an earlier paper, 9 (2), 126-144 (1911), a discussion is given of the convergence of integrals of type  $\int_0^\infty \exp(ix^a) dx / x^b$ .

<sup>4</sup> Ingham, A. E., *Jour. Lond. Math. Soc.*, 1, 34-35 (1926).

# PROCEEDINGS OF THE NATIONAL ACADEMY OF SCIENCES

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## *GROWTH CORRELATES OF ELECTROMOTIVE FORCES IN MAIZE SEEDS*

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Each living maize seed being a physicochemical system has an inherent electrical potential which is measurable after the seed has been soaked for several hours in water. This potential is presumably the sum of the e.m.f.'s of each individual cell, and as such the degree of magnitude of this force may be expected to show correlations with growth processes which are then being initiated. Burr (1943) has already shown a connection between the mean potential of an inbred line and its probable heterotic value in crosses. He further showed the large potential difference implicit in two inbred lines whose large size differences are conditioned by a single gene, the recessive allele in a homozygous condition, resulting in diminution of plant and ear size. This seems to point rather clearly to a positive relationship between the electrical potential and the genetic constitution. Correlations between plant growth and the electrical potential are the easiest way to elucidate this relationship, and it was with this thought that this series of investigations was begun.

The technique employed was as follows. The seeds were placed in tap water for 18-24 hours before measurement. A seed was picked at random after soaking and placed on a cone of plasticene located in the center of a revolving stage (Fig. 1). Silver-silver chloride electrodes in physiological salt solution, terminating in camel's hair brushes, were mounted in a Zeiss manipulator and brought into contact with the micropylar and germinal ends of the seed. The electrodes were connected by flexible unshielded copper wire to the input binding posts of a d. c. microvoltmeter (Burr, 1936). The magnitude of the potential difference was read from a standard galvanometer calibrated to read in millivolts. By means of the revolving stage the seed was rotated 180° and a second determination made. This was repeated until consistent readings were obtained; usually this re-

quired 3 to 5 measurements. A large number of tests were made to determine the validity of the above procedure.

Certain consistencies are encountered in the measurement of the e.m.f. in maize seeds. Measurements can be taken in any one of the three dimensions with a measurement in the longitudinal axis giving the largest potential figure for any given seed. The measurements of e.m.f. from side to side and back to front are always smaller and generally proportional to that of the long axis with the measurement from side to side being usually larger than from back to front.

When e.m.f. measurements are taken on the long axis, it is found that the micropylar end is almost always negative to the germ end of the seed.



FIGURE 1

A photograph of the electrode placement on the maize seed.

Further, if an electrode is placed at the micropylar end, and the other electrode is shifted about the surface of the seed, it is found that there is a regular pattern of equipotential lines over the surface (Fig. 2).

Moreover, it is found that two e.m.f. measurements can be taken on any given seed after soaking, and that both of these measurements can be shown to be correlated with certain measurable attributes of later plant development. The first potential is the reading obtained when the electrodes first touch the seed. The galvanometer reading must be taken immediately for the needle falls off rapidly until within thirty to one hundred and twenty seconds it has attained a stable point at which point it will remain constant for two to five minutes. This falling off in potential and



subsequent attainment of a fixed potential is presumably due to an ionic equilibrium between the seed and the conducting solution of NaCl. This second potential is designated as the equilibrium potential, and is the reading on which Burr based his ideas of the correlation between electrical potential and genetic constitution. The first or unstable potential reading will be designated as the prime potential.

*Prime Potential.*—In the spring of 1945, a five-by-five latin square test was set up in which the five entries were taken from a commercial sample of double-crossed field corn hybrid, U. S. 13, on the seeds of which the prime potential was taken. The entries in the test were: (1) low prime potential, (2) medium prime potential, (3) untested seed, (4) high prime potential and (5) untested seed.

The seed of this test was planted directly in the field with a single seed being planted at each place. Analysis of the results shows that there was a highly significant difference in germination between entries (table 1).

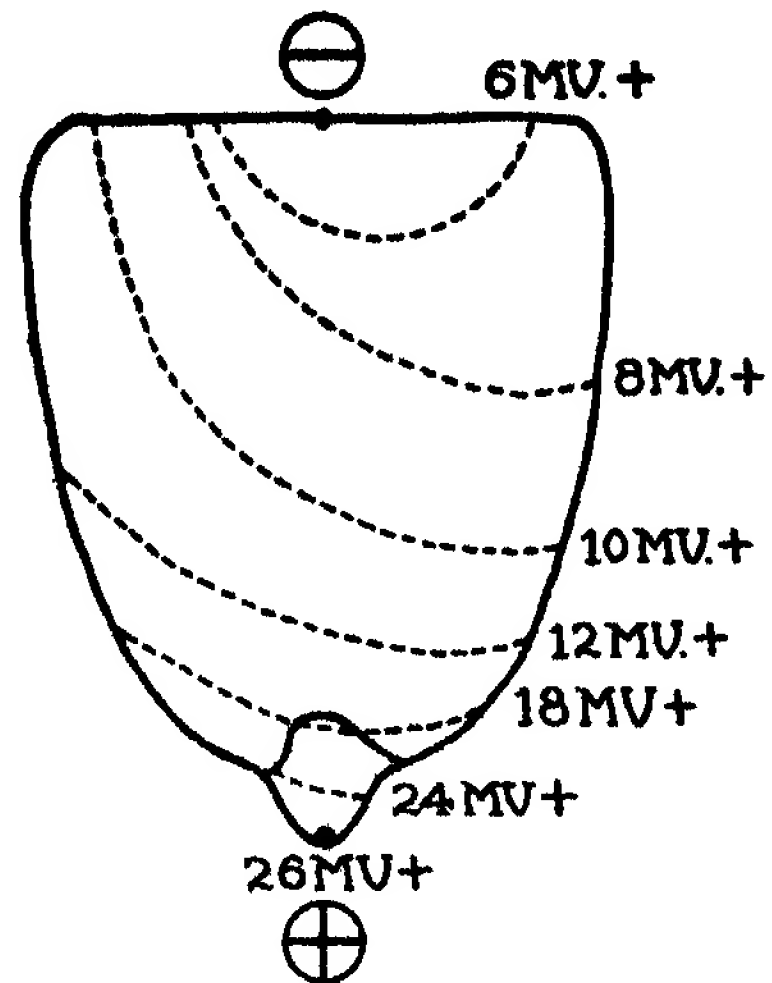


FIGURE 2

Equipotential lines on the surface of the maize seeds.

TABLE 1  
ANALYSIS OF VARIANCE FOR % GERMINATION (PRIME POTENTIAL TEST 1945)

SOURCE OF VARIATION	DF	MS	F
Rows	4	285.86	4.30*
Columns	4	67.06	1.01
Potential level	4	579.76	8.76†
Error	12	66.44	
Total	24		

\* Significant on 0.05 level.

† Significant on 0.01 level.

Table 2 gives the percentage of germination for each entry. The high potential had a significantly higher percentage of germination than the low potential while the medium potential and the untested entries are much alike in percentage of germination and are also significantly higher than the low potential level. Table 2 also shows the yield figures in grams/100 for the five entries. Here again the high potential level and the two un-

tested entries are significantly higher than the low potential level. But the table also shows the obvious connection between the percentage of germination (and hence the plant number) and the yield for an entry. When the yield is corrected for discrepancies in stand by covariance (Singleton and Nelson 1945), these differences are all obviated showing that only the differential germination affected yield and nothing inherent in the entries themselves.

TABLE 2

GERMINATION PERCENTAGES AND YIELD IN GRAMS/100 FOR PRIME POTENTIAL LEVELS

ENTRY	% GERMINATION	YIELD
1 (Low potential)	63	211
2 (Medium potential)	79	242
3 (Untested)	83	269
4 (High potential)	92	277
5 (Untested)	85	271
J. S. D. on 0.01 level,	16	55

In addition to germination, the heights of the plants were measured at weekly intervals throughout the season, and silking and tasselling dates were taken. There was practically no difference in silking and tasselling dates between entries. At no time during the season were the heights of the various entries more than minutely different.

This evidence seems to point to the conclusion that the prime potential is a measurement indicative of seed quality, irrespective of genetic constitution. The seeds from the high potential level will germinate significantly better than those from the low potential level but an advantage is not apparent even in seedling vigor once the plants have emerged from the ground, and no variations other than differential germination can be shown between the entries.

*Equilibrium Potential.*—The stable measurement characteristic of a given seed after it has been in contact with the electrodes for thirty seconds up to two minutes is almost invariably smaller than the prime potential. Just how closely it approximates the prime potential varies from seed to seed and in a larger sense from variety to variety.

In 1943 equilibrium potentials were measured on nine inbred and hybrid lines of corn. The mean measurements and range for each line are given in table 3.

The comparative potentials of the inbred and hybrid lines are in agreement with Burr's findings that the potential of a hybrid between two inbreds is nearly always larger than the potential characteristic of the inbred with the smaller potential, and may be larger than the potential of either parent. Unfortunately, the inbred Ohio 40B which enters into two of the combinations was not tested or grown, and it is rather difficult to explain the excess of potential difference of 40B  $\times$  38-11 over 40B  $\times$  L317 since

L317 itself has a significantly higher potential than 38-11. However, if the potential measurement is correlated with hybrid vigor, this would be explained since 40B and L317 are both Lancaster inbreds and, in general, would not be expected to show as much hybrid vigor when crossed as a cross of 40B  $\times$  38-11 which are two unrelated inbreds.

The readings of 18 for  $(Wf \times Kr)F_2$  and 15 for  $(Wf \times Kr)F_1$  agree with other comparisons of potentials of  $F_1$  and  $F_2$  seed from the same cross made here at the Connecticut Station. The  $F_2$  seed has a slightly but not significantly higher potential.

Within any line (inbred or hybrid) there is approximately normal distribution of the potential readings about a mean. Seed of these nine entries was divided into high, low and medium potential within each entry. This division served as a basis for replication in a randomized block test since it was not believed that potential differences within entries would be significant. In the following results we have eliminated the medium division of each entry from consideration since retention of this division leads to a constant intergradation of potential reading for the entry where we desire two clearly defined groups.

TABLE 3  
MEAN EQUILIBRIUM POTENTIAL IN MILLIVOLTS—1943

	$\times$ POTENTIAL	RANGE
Ind. 38-11, 1941 seed	17	9-26
Iowa L317, 1941 seed	27	7-52
38-11 $\times$ L317, 1942 seed	21	5-35
L317 $\times$ 38-11, 1941 seed	22	3-45
38-11 $\times$ L317, 1941 seed	32	15-50
Ohio 40B $\times$ 38-11, 1942 seed	39	12-60
Ohio 40B $\times$ L317, 1942 seed	28	11-44
$(Wf \times Kr)F_2$ , 1941 seed	18	6-46
$(Wf \times Kr)F_1$ , 1941 seed	15	2-35

In the field test there was no significant difference between high and low divisions of any entry for percentage of germination. The germination percentage for all highs was 87.8 while for all lows it was 83.2, the difference being 4.6. Measurements were taken of the height of plants at weekly intervals throughout the season and figures 3-5 show the plant height in inches for both high and low divisions of each entry plotted against the time in days. There is also a summation in the tenth graph with plant heights averaged for all highs and all lows plotted against time in days. It is evident that in seven of the nine entries, there was a noticeable tendency for the highs to grow faster and attain a greater height by the end of the growing season with the segregation of high and low growth rates occurring early in the season and exactly corresponding with the high and low potential reading. In the other two entries there was a segregation of high

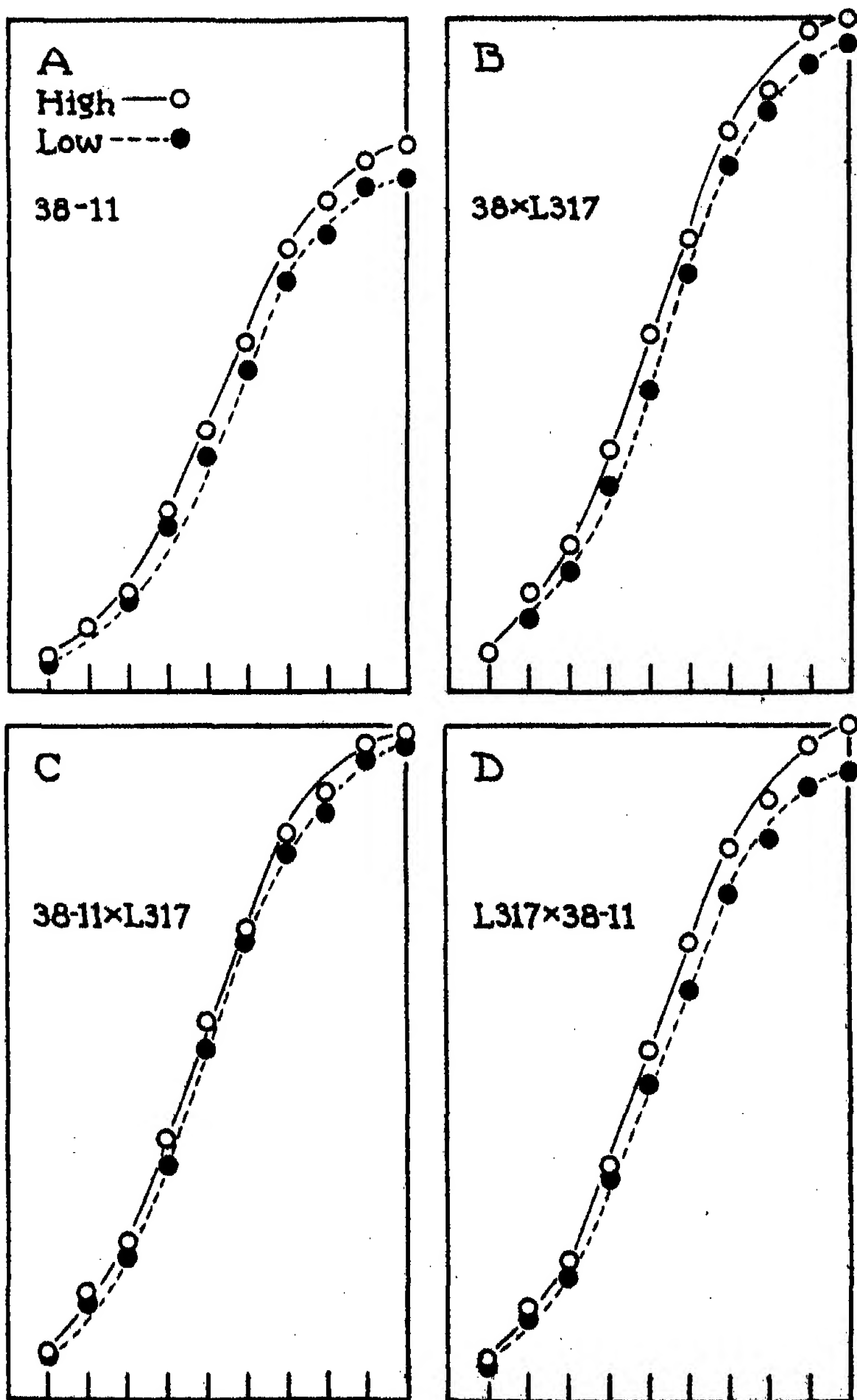


FIGURE 3

Graphs of the height of corn plants at weekly intervals from 4 measured strains.

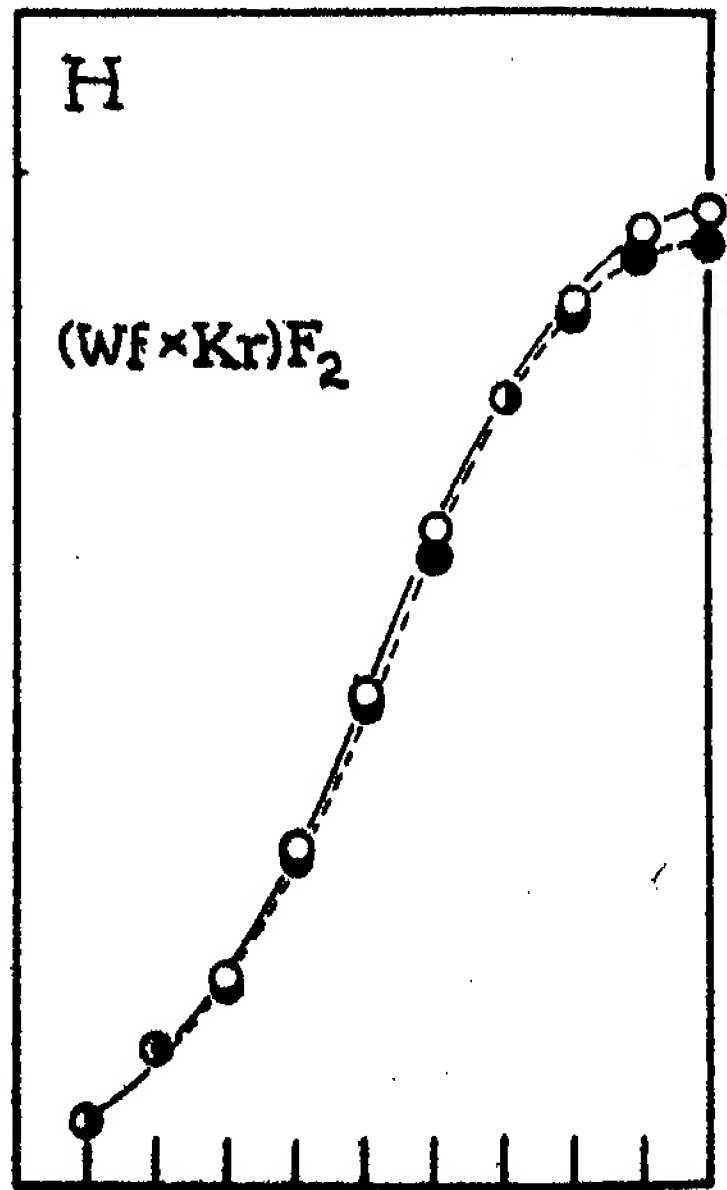
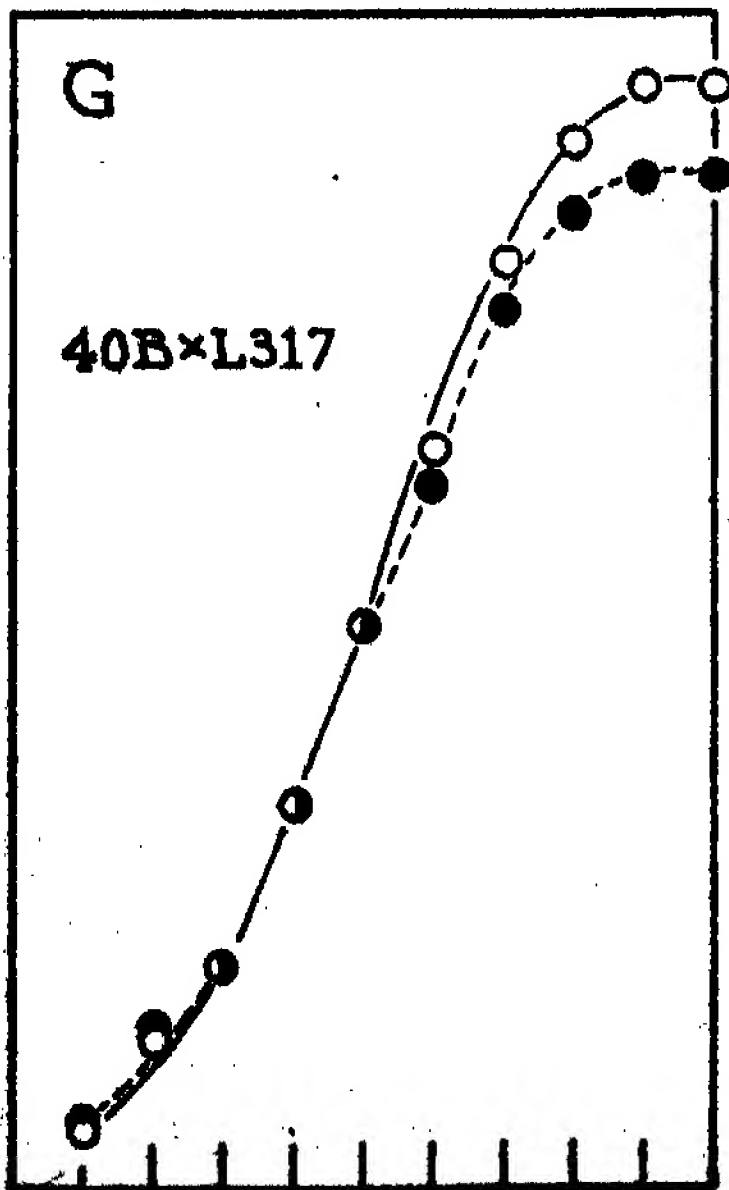
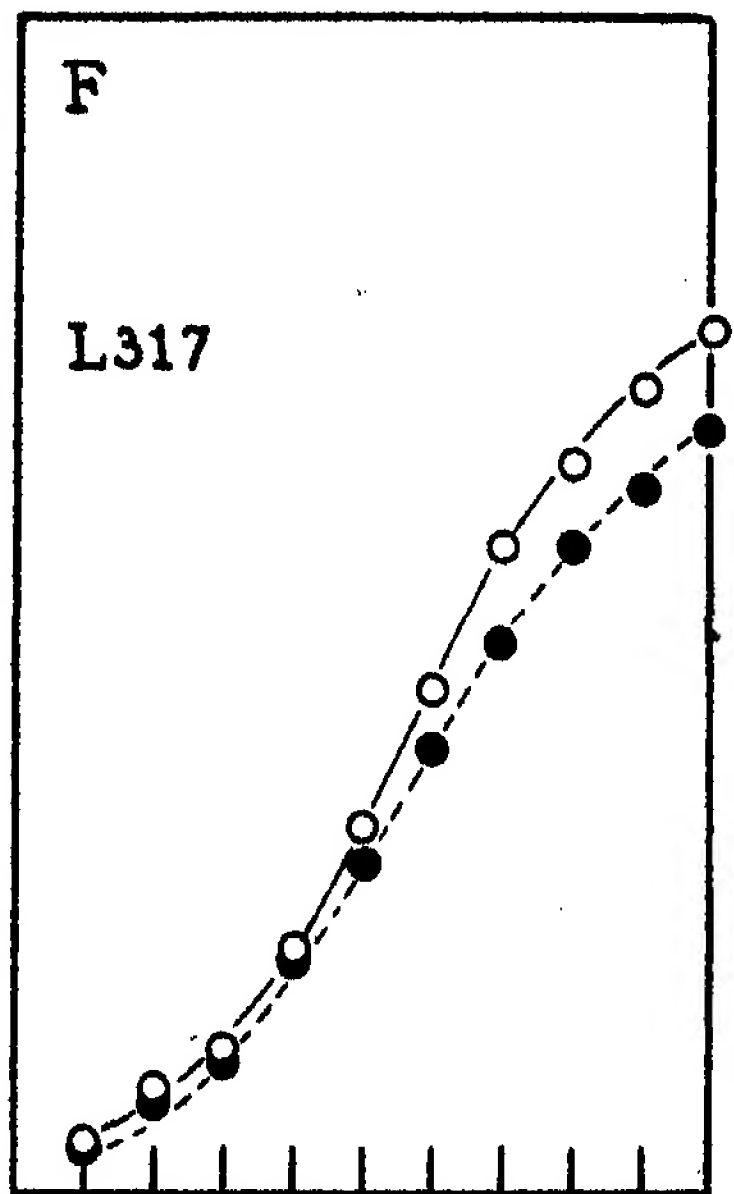
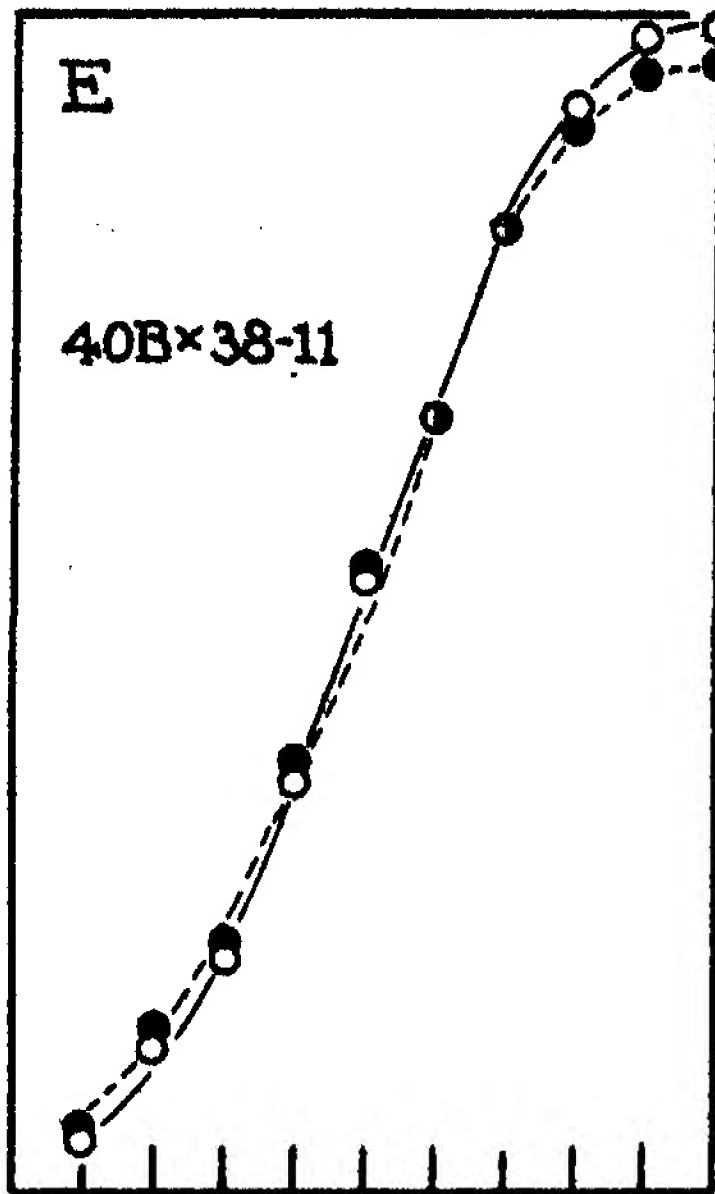


FIGURE 4

Graphs from 4 additional lines.

and low growth rates but not until late in the season. The noteworthy point in this instance is that both these entries, where there was no segregation of growth rates until late in the season, have an inbred, Ohio 40B, in common. By the end of the season in every case, the plants which came from high potential seeds had grown the tallest (table 4).

TABLE 4

HEIGHT IN INCHES OF HIGH AND LOW DIVISIONS OF EACH ENTRY—1943

	HIGH	LOW	HIGH-LOW
38-11	97	91	6
L317	88	77	11
38-11 × L317, 1942 seed	120	116	4
L317 × 38-11	120	112	8
38-11 × L317, 1941 seed	119	116	3
40B × 38-11	119	115	4
40B × L317	113	106	7
(Wf × Kr) $F_2$	100	96	4
(Wf × Kr) $F_1$	108	104	4

$$t = 6.54 \quad P < 0.001$$

If the true population mean were zero, indicating no difference in height between the high and low groups, a mean difference as large as that observed would be expected to occur by chance alone less than once in 1000 similar trials. Further, the probability that by chance alone all nine values in the high-low column will be of the same sign as they are in this instance are only two in 512.†

Table 5 shows similar results for yield in pounds per plot for each entry.

TABLE 5

YIELD IN POUNDS PER PLOT FOR HIGH AND LOW DIVISION OF EACH ENTRY—1943

	HIGH	LOW	HIGH-LOW
38-11	1.4	0.6	0.8
L317	0.7	0.3	0.4
38-11 × L317, 1942 seed	11.4	6.1	5.3
L317 × 38-11	11.4	9.8	1.6
38-11 × L317, 1941 seed	11.5	8.3	3.2
40B × 38-11	11.0	9.4	1.6
40B × L317	9.0	10.3	-1.3
(Wf × Kr) $F_2$	7.0	3.1	3.9
(Wf × Kr) $F_1$	8.4	8.5	-0.1

$$t = 2.429$$

For  $n = 8$ , only one value in twenty will exceed 2.306 by chance alone. It is, therefore, apparent that separating the seeds of any given strain into a high and low group by their equilibrium potential has in the main been effective in placing the plants of superior growth characteristics in one category and the plants of inferior growth characteristics in the other category. Perhaps the e.m.f. measurement is a rough index of the physiological ef-



iciency of a given genotype. In this connection it is rather unexpected that the divisions in inbred lines should as be divergent in growth as the L317 and 38-11 lines tested here. However, Jones (1945) has found unexpected variability in inbreds after many years of inbreeding and indications of heterotic vigor in sublines when crossed together. These appeared as morphological mutants in segregating and homozygous progenies. Presumably there are also subvisible mutants, the manifestations of which are limited to slowing the growth rate of the plant and lessening total growth.

In 1944, three lines were subdivided on the basis of potential difference and grown in separate latin square field tests for each line. Number 1 was a very uniform field corn inbred C20, the second was the  $F_2$  of a cross of two field corn inbreds ( $Wf \times Hy$ ), the third was the  $F_2$  of a cross of a field corn inbred C243  $\times$  1062 (a genetic tester for chromosome 1 and carrying  $br/br$ ,  $bm_2/bm_2$ ,  $f_1/f_1$ ).

The seeds for each entry were weighed after the test for potential differences, and weight of seed was found not to be correlated with potential level. Germination was recorded and found to be equal for all potential levels. However, all growth and yield records from the summer of 1944 must be discounted. There was a departure from normal precipitation of  $-9.65$  during the months of May, June, July and August making the season so dry that plants made very poor growth and produced only about one fifth their normal yield. Nevertheless, Test No. 3 with the  $F_2$  of crosses involving  $br$ ,  $f_1$  and  $bm_2$  contributed useful information. Since the stock was segregating for these three recessive characters (one of which,  $br$ , is definitely deleterious since it dwarfs the plants by shortening all the internodes) it was suggested as a possibility that perhaps all the brachytic, or brachytic and brown mid-rib, might be concentrated in one potential level. This was not substantiated in field tests for the various genes and combinations of genes seemed to be distributed at random in the various levels (table 6).

TABLE 6  
PERCENTAGE OF PLANTS SHOWING RECESSIVE CHARACTERS IN EACH POTENTIAL LEVEL

GENE	HIGH	LOW
$bm_2$	30	32
$br$	23	16
$f_1$	8	5
$bm_2 + br$	10	13
$bm_2 + f_1$	8	5
$br + f_1$	8	5
$bm_2 + br + f_1$	5	5

It was possible to obtain selfed seed on various potential levels in the No. 2 test( ( $Wf9 \times Hy$ ) $F_2$ ). Two lots of seed were selected at random from the selfed ears of the low potential plants and two from the seed of the high

potential plants. Potential levels had been measured in the  $F_2$  seed in 1944, and the  $F_3$  seed was considered to belong to the same potential levels without further testing. It was immediately apparent that there were considerable differences between the rows coming from the selfed seed of high potential plants and those coming from seed of low potential plants. The high potential plants were taller at every point during the growing season and in general were sturdier and leafier. Figure 5 gives a growth curve of height in inches plotted against time in days for the two high rows combined and the two low rows combined, and it seems certain that the progeny of high potential plants started faster and grew larger. Although results were combined in figure 5 it is well to mention that two high rows were very much alike in growth and superior to either low row.

We now have the intriguing situation in which e.m.f. differences found in the  $F_2$  seeds are very markedly correlated with the growth characteristics of the  $F_3$  plants. At the same time what has happened to the actual e.m.f. in the stocks between the  $F_2$  and the  $F_4$  which is the seed we now have on hand. The high division for the  $F_2$  seed comprised those seeds with potentials of 17–30 millivolts while the low division had seeds with 0–2 millivolts as tested in 1944. After the large growth differences were obtained in 1945, the available  $F_3$  and  $F_4$  seed was tested. One selfed ear ( $F_3$ ) from a high potential row had a mean of 34.20 millivolts while  $F_4$  seed from this same ear measured 35.0. The  $F_2$  seed from a low potential tested 26.1 millivolts, and no  $F_4$  was available for measurement. This represents a considerable rise in  $F_3$  and  $F_4$  potentials over the  $F_2$  for which it is difficult to account. However, the  $F_2$  stock would be highly heterogeneous and capable of very considerable segregation of superior growth factors.

In connection with the differences between prime and equilibrium potential as reflected in percentage of germination, seed of the same sample of U. S. 13 as used in the prime potential tests (1945) was divided on the basis of its equilibrium potentials into high, medium and low groups. Two groups of untested seed were added to give five entries in a randomized block field test. There were 3 replications in this test which was intended solely to check germination. The percentage of germination for each entry was: untested (1) 93%, high 93%, untested (2) 93%, low 91%, medium 93%. The higher over-all percentage of germination was due to this test being planted in the middle of the summer with much less severe conditions than prevailed for the other test. But it serves to bear out other observations that the percentage of germination and the equilibrium potential are not correlated.

*Summary.*—For any maize there are two possible potential determinations. The first of these, the prime potential, is apparently highly correlated with seed viability, but with no other measured attribute of plant growth. The second of these potentials, the equilibrium potential, is not

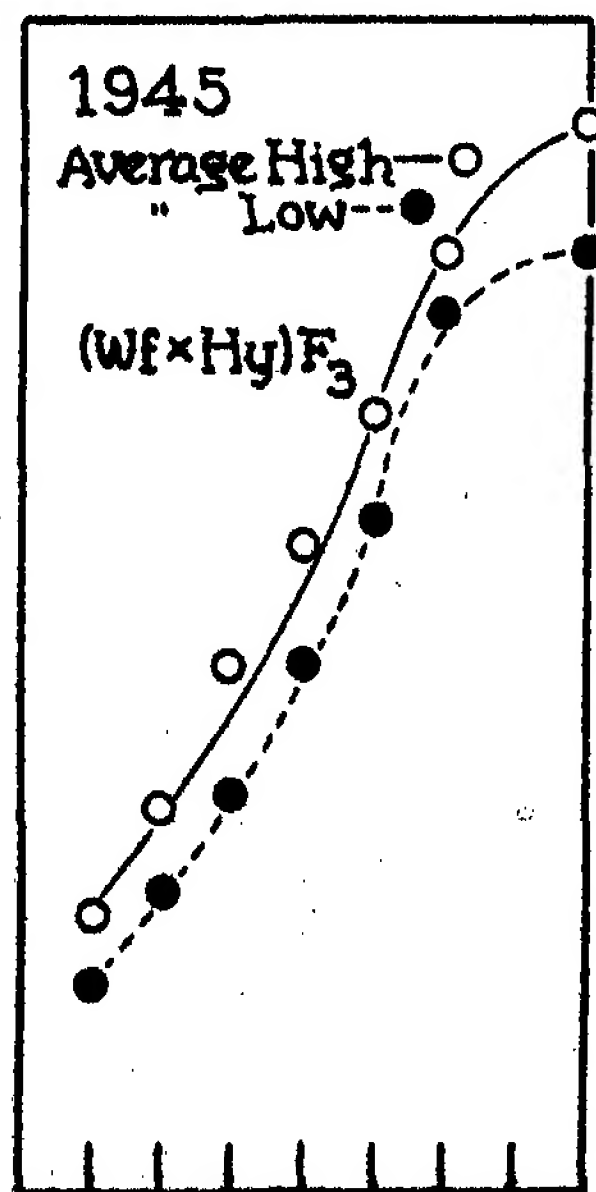
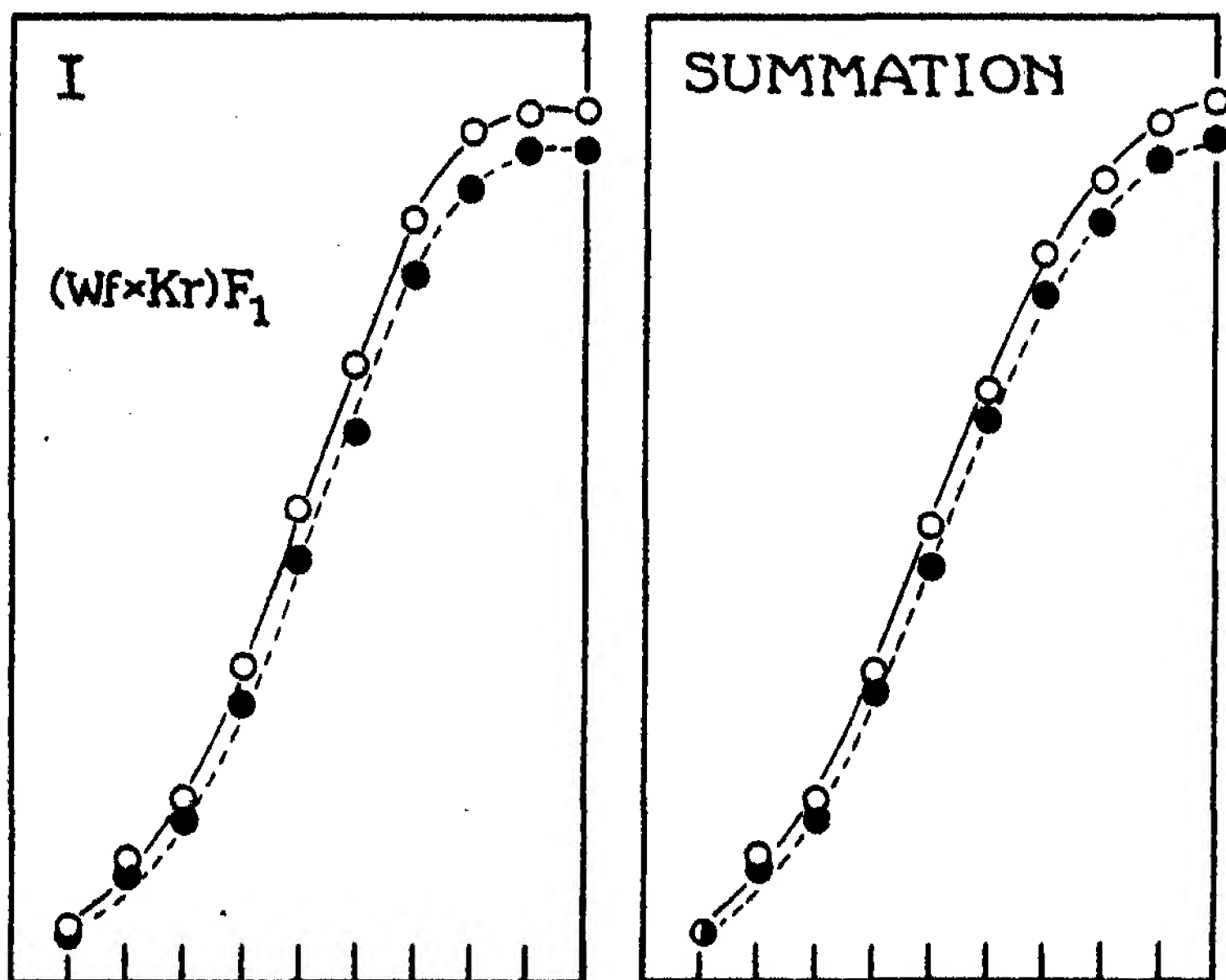


FIGURE 5

Graph of one additional line, plus a summation curve for all lines. Also growth curves from plants from  $F_2$  plants where potential measurements were made on the  $P_2$  seed.

correlated with seed viability but rather with the inherent genetic constitution of a plant since by use of the potentiometer and equilibrium potential determinations, one is enabled to segregate from a given population those seeds with superior growth characteristics. Further, these potential differences between seeds have been highly correlated with the growth of progeny which were one generation removed. For these reasons, the potentiometer may prove to be a useful tool for plant breeders.

\* Graduate Fellow of the Eastern States Farmers' Exchange.

† The aid of Dr. C. I. Bliss is gratefully acknowledged.

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INTERSEXES DEPENDENT ON A MATERNAL EFFECT IN  
HYBRIDS BETWEEN *DROSOPHILA REPLETA* AND  
*D. NEOREPLETA*

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*Drosophila repleta* Wollaston and *D. neorepleta* Patterson and Wheeler are closely similar species, the former widely distributed and the latter known from Guatemala. It was found by Dr. E. Novitski that these species occasionally cross, and that the  $F_1$  females sometimes give a few offspring when mated to repleta males (see Wharton 1942 and Sturtevant 1946).

I have found a sex-linked recessive white-eyed mutant type in *D. repleta* (actually not quite white, but retaining only a slight tinge of color). At least 5000 neorepleta females have been crossed to white repleta males (in a few cases the repleta males carried singed, another sex-linked recessive, rather than white). These matings included at least 500 mass cultures, of which 74 produced hybrid offspring—a total of 532 females and 635 males, all wild type for the sex-linked mutant characters used. The males had very narrow testes, and were wholly sterile. The females were variable; most of them had bristles somewhat reduced in size ("minute"), and many of them had three anal plates instead of the usual two—this last character suggesting intersexuality.

About 400 of these  $F_1$  females were mated to white repleta males, and offspring were obtained from 34 of them—the total output being 70 wild-type females, 9 white females, 42 wild-type males and 58 white males. The expectation is for these classes to be equal; there is evidently a great

deficiency of white females. Of the 9 recorded in this class, notes made on two make it possible to be certain (in the light of later results, to be described below) that they were intersexes, and it is possible that some of the other 7 were also of this nature, as were probably a few of the white males.

The wild-type males from this first backcross resembled the  $F_1$  males in having long narrow testes and in being sterile. The wild-type females, however, included some moderately fertile individuals, which were again crossed to white repleta males. Such successive backcrosses of wild-type females to white repleta males have now been continued through many generations, and have resulted in strains presumably pure repleta in composition except for a section of the  $X$ -chromosome near the locus of white. In some of these strains the double recessive, white singed, has been used instead of white; and in these strains all the wild-type males are sterile and have narrow testes, while all the white-singed males have normal testes and are as often fertile as are white-singed males with no neorepleta chromosomes in their immediate ancestry. Tests of the few crossovers between white and singed indicate that the locus of the narrow-testis gene (or genes) lies between white and singed, and very near white.

The wild-type females from the backcrosses, when mated to white repleta males, appeared to fall into two classes; some of them gave the four types of offspring in approximately equal numbers, whereas others continued to give a marked deficiency of white daughters. The daughters of the first type of female always repeated the first type of result; but the females of the second type commonly yielded few offspring, and their very existence as a distinct type was at first uncertain because of the confusing effects of sampling errors in the small families obtained from them. Finally, however, after four successive backcrosses, a single female of this type was obtained which was more fertile, and her descendants have retained this fertility. Presumably the neorepleta gene responsible for the unusual ratios was at first linked to another gene that decreases fertility in females largely repleta in constitution, and in this case the infertility gene was lost by crossing over. The later studies have all been carried out with descendants of this more fertile female. There are, however, enough fragmentary data to make it clear that the behavior is essentially the same in lines derived from other  $F_1$  hybrids—i.e., the results are really due to genes derived from neorepleta, rather than to a mutation that occurred in this line.

From females of this more fertile line, backcrossed to white repleta males, a total of 33 wild-type daughters (of females giving a deficiency of white daughters) have been tested by white males. Of these, 16 gave approximately 1 + ♀ : 1 w ♀ : 1 + ♂ : 1 w ♂; the remaining 17 all gave a deficiency of white females. The total counts from these 17 were: 472 + ♀, 5 w ♀, 63 w intersexes, 482 + ♂, 339 w ♂. The 5 white females pre-



sumably represent crossing over between the loci of white and of the critical gene in the *X* derived from neorepleta. Their nature can be more accurately determined when other sex-linked genes are introduced in the crosses; it is, however, already clear that the gene concerned is not the same as that responsible for the narrow testes of the hybrid males.

The intersexes are of an extreme type, with gonads very small (rudimentary ovaries in those cases where they were found at all); external genitalia missing or of abnormal male type; wings usually not expanded, and, when they are, usually with thickened veins; one or more (sometimes all four) scutellar bristles often absent. They are weak individuals—and evidently usually die before emergence. It is to be supposed that such preimaginal mortality is responsible for the difference in number of wild-type females (472) and white intersexes (63). While no systematic study has been made, white-eyed flies have been found dead in their puparia in such cultures.

These results suggest that there is an autosomal dominant gene, derived from neorepleta, that so conditions the eggs (before meiosis) that two repleta *X*-chromosomes result in the development of intersexes rather than females. Evidently the action comes before meiosis, and the autosomal gene in question may be absent in the intersexes themselves. This interpretation has been confirmed by tests of the white brothers of intersexes. Such males, when crossed to pure repleta females, gave normal offspring of both sexes; but some of their daughters (presumably half of the daughters from half of these males, though the data are not extensive enough to establish this), when mated to repleta males, gave only intersexes and males.

It will be seen that this last experiment shows that the intersexes are not dependent on the presence of neorepleta cytoplasm, since their mothers were offspring of pure repleta females. The experiment also shows that the autosomal gene from neorepleta has no phenotypic effect on females that have received it from their father. The result likewise agrees with the earlier ones in indicating that an individual with two repleta *X*'s is intersexual whether or not it carries this gene, provided the gene was present in its mother.

*Conclusions.*—*D. neorepleta* carries an autosomal gene which, when present in single dose in a hybrid female, makes her eggs male in potentiality. This predisposition to maleness is only partially overcome by two repleta *X*'s, and male-like intersexes result. One repleta *X* and one neorepleta *X* are sufficient to produce normal females. This autosomal gene is present in two doses in pure neorepleta eggs. Two neorepleta *X*'s are sufficient to cause such eggs to develop into normal females; but in the *F*<sub>1</sub> hybrids, having one neorepleta *X* and one from repleta, some of these eggs develop into females with male-like anal plates.



Presumably the essential sex-determining mechanism is the same in the two species, but both the male-determining action of the autosomal gene and the female-determining action of the *X* are stronger in *neorepleta*.

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Wharton, L. T., "Analysis of the Repleta Group of *Drosophila*," *Univ. Texas Publication* 4228, pp. 23-52 (1942).

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## THE MECHANISM OF POSITION EFFECT—EXPERIMENTS ON THE PHENOTYPIC EXPRESSION OF POSITION EFFECTS IN RELATION TO CHANGES IN PAIRING OF NEIGHBORING CHROMOSOME REGIONS

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In a previous paper<sup>1</sup> we discussed two alternative types of interpretation of the phenomenon of position effect, and expressed a predilection for one of these alternatives, partly on the grounds that it seemed to lend itself more readily than the other to experimental tests. The hypothesis as to the mechanism of position effect that we thereupon elaborated was closely related to some early suggestions of Muller.<sup>2, 3</sup> Briefly stated, it postulated the following chain of events. In an organism such as *Drosophila*, where somatic pairing occurs, chromosomal aberrations change the pairing relationships of the chromosome regions adjacent to the breaks, or facing the breaks. The forces which bring about pairing may thus achieve a new distribution on either side of a gene located near to or facing a break. Such a change in pairing forces might subject this gene to a changed condition of stress. Now, if we visualize the gene as a complex folded protein molecule, or part of such a molecule, the specific activity of which is determined by the spatial configuration of specific groups on its surface, then a change of stress might be expected to lead to a change in the degree of extension of the folded protein, hence to a change in the spatial relationship of the active group, and so finally to a change in the specific activity of the gene, which change may be manifested phenotypically as a position effect.

We concluded that if this hypothesis were valid it should be possible, given an already existing position effect, to modify its phenotypic expression by further changing the pairing relationships of the chromosome regions in the immediate neighborhood of the affected gene. Moreover, it seemed clear that it would not be necessary for this purpose to alter the

already aberrant chromosome in which the affected gene itself was located, but that the change in pairing, and therefore the phenotypic change, should be obtained equally well by introducing an aberration in the homologous chromosome, opposite and close to the affected gene.

The experiments presented here were designed to serve this purpose.

*Experiments and Results.*—For the position effects, the white ( $w$ ) locus was chosen, and three white-mottled stocks ( $w^{m4}$ ,  $w^{m5}$  and  $w^{258-18}$ ) were used.<sup>4</sup> The first of these ( $w^{m4}$ ) involves an inversion in the  $X$ -chromosome, with one break to the left of  $w^+$  and the other in the heterochromatic region of the chromosome, while each of the other two stocks carries a translocation between the  $X$ -chromosome and chromosome 4, the break in  $X$  being to the right of  $w^+$ . In all three stocks the males and homozygous females are viable, and the white-mottled eyes are characteristic of these flies as well as of females heterozygous for the rearrangement and for a normal  $X$  carrying the mutant allele  $w$ .

In order to combine the position effects with an aberration adjacent to the  $w$  locus in the opposite chromosome, deficiencies were used, and a comparison was made of heterozygous females ( $w^m/y Hw w$ ) and females ( $w^m/Df w$ ) in which an  $X$ -chromosome with a deficiency next to the  $w$  locus was substituted for the  $y Hw w$  chromosome, the dominant  $Hw$  being used as a marker to distinguish the two types (see figure 1; here, and throughout this paper, the following symbols are used:  $w$  = white,  $w^m$  = white-mottled,  $y$  = yellow,  $Hw$  = hairy wing,  $Df$  = deficiency).

Three deficiency stocks were used,  $w^{258-45}$ ,  $w^{258-48}$  and  $w^{258-14}$ , in which there are deficiencies of one band, 5 bands and 13 bands, respectively, immediately to the left of, but not including, the  $w$  locus (assumed to be associated with band 3 C 2.3)<sup>4</sup> which was represented by the recessive  $w$ .

Crosses of the general type  $Df w/y Hw w \text{ } \varnothing \times w^m \text{ } \sigma^7$  were made, so that the  $w^m/Df w$  and  $w^m/y Hw w F_1$  females to be compared developed in the same bottle under the same conditions. Flies were raised at 25°C. except in a few of the initial experiments, which were run at variable room temperatures, probably averaging 26° or 27°C.

The  $w^m/Df w$  flies and the  $w^m/y Hw w$  controls were classified individually as to eye-color in four arbitrary groups—light, light intermediate, dark intermediate and dark. As there was no objective control (such as a color chart) for this system of classification, the standards adopted for the four groups may have varied somewhat from time to time. Nevertheless, the method was satisfactory for comparing flies at any one time, and the conclusions drawn from comparison of  $w^m/Df w$  flies and  $w^m/y Hw w$  controls from the same bottle are valid.

In eight out of the nine possible combinations between the three white-mottled and the three deficiency stocks, a significant difference<sup>5</sup> was observed in the distribution of the  $w^m/Df w$  and  $w^m/y Hw w$  flies in the four

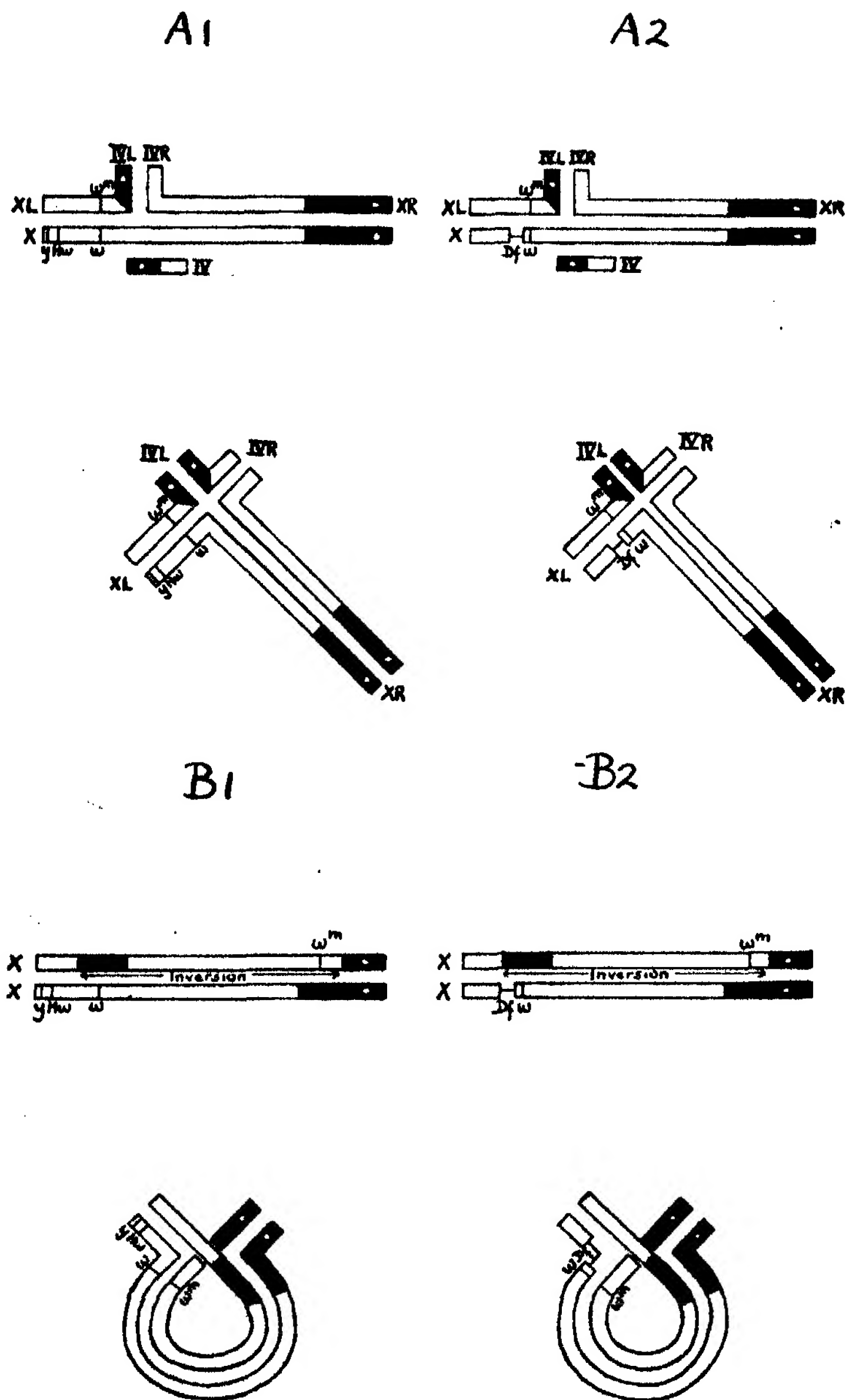


FIGURE 1

Diagram showing *X*-chromosomes (first and third lines) and their pairing (second and fourth lines). *A* represents combinations with the *w*-mottled stocks *w<sup>m1-12</sup>* and *w<sup>m4</sup>*; *B*, combinations with the *w<sup>m4</sup>* stock. *A1* and *B1*, controls; *A2* and *B2*, substitution of an *X*-chromosome with a deficiency (single line) for a normal *X*. Maximum pairing is assumed for euchromatic regions (double line), but the non-homologous pairing of heterochromatic regions (solid black) is not indicated.



thus closely linked with  $w$ ) and affecting the total balance of  $+$  and  $-$  modifying genes through their presence or absence; or (c) closely linked with  $w$ , but not in the region of the deficiency.

2. The differences could be due to differences in competitive action of the  $w$  alleles in the deficient chromosomes, like that postulated by Stern<sup>6</sup> in the case of *cubitus interruptus* alleles.

These possibilities will be considered in order.

1. (a) To make all stocks as isogenic as possible, they were all outcrossed repeatedly to a single stock of  $y Hw w$ . The crosses were also made in such a way as to select for viability of the  $w^m/Df w$  combinations, which in some cases was rather poor. The procedure adopted was to obtain each  $w^m/Df w$  combination, outcross it to  $y Hw w$  males, and in the next generation breed together  $Df w/y Hw w$  females and  $w^m$  males. Then the  $w^m/Df w$  females were selected and outcrossed to  $y Hw w$  males a second time, and so on. This procedure should have two results: (1) replacement of autosomes of the  $w^m$  and  $Df w$  stocks by those of the  $y Hw w$  stock, and (2) replacement by crossing-over of parts of the  $Df w$  and eventually of the  $w^m$  X-chromosomes by the  $y Hw w$  X. Although the  $y Hw w$  stock itself was not strictly isogenic, it should thus serve to give both  $w^m/Df w$  and  $w^m/y Hw w$  flies a similar range of variation in genetic background, with the added reservation that in regions close to the breaks the substitution of genes from the  $y Hw w$  stock could not be expected at all, owing to the low frequency of crossovers in these regions.

The extent to which different combinations were outcrossed and the results of the outcrossing, are shown in table 2. The most important of these results is that of the  $w^{m5}/w^{258-14}$  combination. A comparison of tables 1 and 2 shows that though at first the eyes of  $w^{m5}/w^{258-14}$  were darker than those of  $w^{m4}/y Hw w$ , after eight generations of outcrossing  $w^{m5}/w^{258-14}$  became lighter than  $w^{m5}/y Hw w$ . This must mean that the original  $w^{258-14}$  X-chromosome carried modifiers for dark eye-color which were effectively removed by crossing-over in the course of several generations of outcrossing to  $y Hw w$ , and that the difference in the opposite direction which then became apparent had been masked by these modifiers at an early stage, before their substitution by genes from the  $y Hw w$  X-chromosome. This later difference therefore cannot be attributed to modifiers in any other part of the X-chromosome than the close neighborhood of  $w$ .

Other combinations which were outbred for several generations continued to show the same difference between the  $w^m/Df w$  and  $w^m/y Hw w$  flies that appeared at the first cross.

In two cases ( $w^{258-18}/w^{258-48}$  and  $w^{m4}/w^{258-48}$  combinations) after crossovers had occurred between the  $Df w$  and  $y Hw w$  chromosome to the left of the deficiency, the crossover chromosome consisting of the left end of the deficiency chromosome (but without the deficiency) and the right end



of the  $y Hw w$  chromosome (without  $y Hw$ ) was tested by making up the combination *crossover*/ $y Hw w$  and mating to  $w^{m4}$  or  $w^{258-18}$  males. The distribution of eye-colors in  $w^m$ /*crossover* and  $w^m/y Hw w$  females of the next generation did not differ significantly, showing that the left end of the deficient  $w^{258-48}$  X-chromosome was not distinguishable from that of the  $y Hw w$  chromosome with regard to modifiers of the position effects.

It may be concluded from the outbreeding experiments that the final differences observed between  $w^m/Df w$  and  $w^m/y Hw w$  flies were not due to any difference in such modifiers as could be separated from the  $w$  region by crossing over. This conclusion is substantiated, as far as the region to the left of  $w$  is concerned, by the tests of crossovers just described.

TABLE 2

EYE-COLORS OF  $w^m/Df w$  FEMALES AS COMPARED WITH THE CORRESPONDING  $w^m/y Hw w$  CONTROLS, AFTER OUTCROSSING TO  $y Hw w$  25°C.

$w^m$ <i>Df w</i> combination	$w^{m5}$ $w^{258-45}$	$w^{m4}$ $w^{258-45}$	$w^{258-18}$ $w^{258-48}$	$w^{m4}$ $w^{258-48}$	$w^{258-18}$ $w^{258-14}$	$w^{m5}$ $w^{258-14}$	$w^{m4}$ $w^{258-14}$
Light	0	0	0	0	0	0	0
Light intermediate	0	1	0	14	0	10	25
Dark intermediate	150	80	2	130	1	99	151
Dark	386	250	64	165	21	222	59
$w^m$ <i>y Hw w</i> control	$w^{m5}$ <i>y Hw w</i>	$w^{m4}$ <i>y Hw w</i>	$w^{258-18}$ <i>y Hw w</i>	$w^{m4}$ <i>y Hw w</i>	$w^{258-18}$ <i>y Hw w</i>	$w^{m5}$ <i>y Hw w</i>	$w^{m4}$ <i>y Hw w</i>
Light	0	0	0	0	1	0	2
Light intermediate	0	0	9	2	29	0	12
Dark intermediate	74	48	98	59	86	38	121
Dark	633	323	156	325	76	355	105
No. of generations outcrossed	10	10	13	14	2	8	7
$\chi^2$	63.3	15.5	31.1	80.5	24.7	60.1	20.2
$n$	1	1	1	2	1	1	2
$P$	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01

(b) The loss of dominant modifiers in the deficient chromosomes could result in a difference in phenotype of flies carrying these chromosomes as compared with those having a complete  $y Hw w$  chromosome. Inspection of the tables, however, shows that the same deficiency (e.g.,  $w^{258-48}$ ) may modify different position effects in opposite directions. In other words, on the assumption of a change in balance of dominant modifiers, we would also have to assume that these modifiers have a different and specific action on different position effects. While this interpretation does not appear probable to us, it obviously cannot be excluded altogether.

There is another possibility, however, namely that the deficiencies uncover recessive modifiers in the opposite chromosome, near to the affected  $w$  locus. In this case, the reaction of different position effects to the same deficiency would be attributable not to specific action of the same



modifiers, but to the presence of different modifiers closely linked with the affected  $w$  loci of the different white-mottled stocks.

If this were so, we would assume for instance that in the  $w^{258-18}/w^{258-48}$  combination, the deficiency uncovers recessive modifiers which darken the eye-color, while in  $w^{m4}/w^{258-48}$  one or more recessive modifiers for lighter color are effective. These modifiers, then, should also be effective in the hemizygous males and homozygous females of the position effect stocks; but this is not the case, for  $w^{258-18}$  and  $w^{m4}$  ♂s were scored after 13 and 12 generations of outcrossing, respectively, and in both cases (not in  $w^{m4}$  only) proved to be lighter than the corresponding  $w^{258-18}/y Hw w$  or  $w^{m4}/y Hw w$  ♀s.

We consider, therefore, that this possibility can be discarded.

It may be added that the probability that differences are due to modifiers in the deficient regions is considerably reduced by the fact that such differences are obtained even with the single band deficiency of  $w^{258-48}$ .

(c) It will be seen that the assumption of very closely linked modifiers outside of the deficiency differing from those of the  $y Hw w$  chromosome again requires the additional assumption of specific and opposite action of these genes on different position effects. We are left in doubt as to this possibility, as in the case of possibility 1(b) above.

2. Stern<sup>6</sup> has obtained results with alleles at the *cubitus interruptus* (*ci*) locus which he interprets on the supposition that some of these alleles compete with one another for the use of a common substrate, and that differences in ability to combine with this substrate or to convert it into a new end-product are the cause of different degrees of phenotypic expression of the *cubitus interruptus* effect in flies carrying different combinations of alleles.

In order to test the  $w$  alleles in our deficiency chromosomes for such a competitive effect, females of the constitution  $w^{258-14}/y Hw w, w^{258-48}/y Hw w$  and  $w^{258-48}/y Hw w$  were mated with apricot ( $w^a$ ) males, and in each case a comparison was made between the  $w^a/Df w$  and  $w^a/y Hw w$  progeny.

It was impossible to distinguish between the two types in any case, so that there is no evidence that any of the deficiencies used have any effect on the expression of the  $w^a$  allele.

*Summary and Conclusion.*—On the assumption that pairing conditions affect the manifestation of position effect, experiments were performed in which the eye-colors of flies, carrying a white-mottled  $X$ -chromosome and either a normal unbroken  $X$  or one with a deficiency next to the  $w$  locus, were compared. The eye-colors appear to be different. While these results can be regarded as supporting the hypothesis which the experiments were designed to test, it cannot be completely excluded at present that they are due to the action of modifiers, or to competition between alleles.<sup>7</sup>

<sup>1</sup> Ephrussi, B., and Sutton, E., these PROCEEDINGS, 30, 183-197 (1944).

<sup>2</sup> Muller, H. J., *Fifteenth Int. Physiol. Congr., Leningrad* (1935).

<sup>3</sup> Muller, H. J., *Cold Spring Harbor Symp. Quant. Biol.*, 9, 151-165 (1941).

<sup>4</sup> Bridges, C. B., and Brehme, K. S., "The Mutants of *Drosophila melanogaster*," *Carnegie Inst. Washington, Pub. 552*, Washington, D. C. (1944).

<sup>5</sup> The  $\chi^2$  test was applied, classes being combined whenever the expected numbers in a single class were less than 5.

<sup>6</sup> Stern, C., *Genetics*, 28, 441-475 (1943).

<sup>7</sup> This paper was read in manuscript by Drs. H. B. Glass, R. F. Kimball, B. McClintock, H. J. Muller and B. H. Willier, to whom we wish to express our appreciation.

## THE DISTORTION OF ANGLES IN GENERAL CARTOGRAPHY\*

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1. *Azimuthal Curves*.—Let a surface  $\Sigma$  be mapped in a point-to-point fashion upon a plane  $\pi$  with cartesian coördinates  $(x, y)$  such that point  $s$  of  $\Sigma$  and  $\pi$  will correspond if they are represented by the same (curvilinear) coördinates. Recently<sup>1</sup> we have introduced the term *cartogram*, to denote any particular mapping of a surface  $\Sigma$  upon a plane  $\pi$ . The cartogram is conformal or general according as the transformation is or is not conformal.

We define the azimuthal ratio  $\alpha$  in the following manner. Let  $d\theta$  denote the angle between two infinitesimally consecutive directions at a fixed point  $p(x, y)$  on the plane  $\pi$ , and  $d\Theta$  the angle between the corresponding consecutive directions at the associated fixed point  $P(x, y)$  on the surface  $\Sigma$ . By the *azimuthal ratio*  $\alpha$ , we shall mean the value of the fraction:  $\alpha = d\Theta/d\theta$ . That is, it is the instantaneous rate of change of the inclination at the point  $P(x, y)$  on the surface  $\Sigma$  with respect to the inclination at the corresponding point  $p(x, y)$  on the plane  $\pi$ .

Our azimuthal ratio  $\alpha$  is a function of the lineal element  $(x, y, y')$ . It is independent of the slope  $y' = dy/dx$  if, and only if, the cartogram is conformal, in which case  $\alpha = 1$ .

An *azimuthal curve* is the locus of a point on the plane  $\pi$  (or on the surface  $\Sigma$ ) along which the azimuthal ratio  $\alpha$  does not vary. In a general cartogram, there are  $\infty^2$  azimuthal curves. (In this connection, conformal cartograms<sup>2</sup> are of no interest since every curve is azimuthal because of the fact that  $\alpha = 1$ .)

In the present article, we shall derive some of the geometrical properties of systems of  $\infty^2$  azimuthal curves. Also we shall compare and contrast these with our properties of scale curves which were developed elsewhere.<sup>3</sup>

2. *The formula for the Azimuthal Ratio  $\alpha$* .—To derive this formula, we

proceed as follows: The square of the linear element  $dS$  on the surface  $\Sigma$ , is

$$dS^2 = E(x, y)dx^2 + 2F(x, y)dxdy + G(x, y)dy^2, \quad (1)$$

where  $H^2 = EG - F^2 > 0$ . On the other hand, the square of the linear element  $ds$  on  $\pi$  is:  $ds^2 = dx^2 + dy^2$ .

The angle  $\Delta\theta$  between any two directions  $y'_1$  and  $y'_2$  at the fixed point  $P(x, y)$  on the surface  $\Sigma$  is given by the equation

$$\tan \Delta\theta = \frac{H(y'_2 - y'_1)}{E + F(y'_1 + y'_2) + Gy'_1y'_2}. \quad (2)$$

The angle  $\Delta\theta$  between the two corresponding directions  $y'_1$  and  $y'_2$  at the associated fixed point  $p(x, y)$  on the plane  $\pi$ , is given by the formula:  $\tan \Delta\theta = (y'_2 - y'_1)/(1 + y'_1y'_2)$ .

Upon dividing the two preceding equations defining the increments of angles and then letting the slope  $y'_2$  approach the slope  $y'_1$ , we obtain the following formula for the azimuthal ratio

$$\alpha = \frac{d\theta}{d\theta} = \frac{H(1 + y'^2)}{E + 2Fy' + Gy'^2}. \quad (3)$$

This formula gives the rate of distortion of the angle in any general cartogram.

Noting that the scale  $\sigma$  is defined by  $\sigma = ds/dS$ , it follows that the azimuthal ratio  $\alpha$  is the product of  $H$  by the square of the scale  $\sigma$ . That is,

$$\alpha = H\sigma^2. \quad (4)$$

3. *The Differential Equation of Second Order Defining the System of  $\infty^2$  Azimuthal Curves.*—Along any curve of this system,  $\alpha = \text{const.}$  Thus upon eliminating the constant  $\alpha$  from the equation (3) by differentiation, we find that the differential equation of our  $\infty^2$  azimuthal curves is

$$y'' = \frac{(1 + y'^2)[E_x + (E_y + 2F_x)y' + (2F_y + G_x)y'^2 + G_yy'^3 - \frac{1}{H}(H_x + y'H_y)(E + 2Fy' + Gy'^2)]}{2[-F + (E - G)y' + Fy'^2]}. \quad (A)$$

It follows that not every system of  $\infty^2$  curves can represent the azimuthal curves of a cartogram since  $y''$  is of special algebraic character in the first derivative  $y'$ .

4. *Comparison of the Systems of Azimuthal and Scale Curves.*—A scale curve is the locus of a point along which the scale  $\sigma = ds/dS$ , does not vary. The differential equation of the  $\infty^2$  scale curves is

$$y'' = \frac{(1 + y'^2)[E_x + (E_y + 2F_x)y' + (2F_y + G_x)y'^2 + G_y y'^3]}{2[-F + (E - G)y' + Fy'^2]}. \quad (S)$$

Upon contrasting the system (A) of azimuthal curves and the system (S) of scale curves, we observe that they are of the same general algebraic structure in  $y'$ .

*The cuspidal directions (corresponding to zero radius of curvature) for both systems (A) and (S) are along the Tissot characteristic net*

$$Fy'^2 + (E - G)y' - F = 0. \quad (5)$$

This is the unique orthogonal net on the plane  $\pi$  which by the general cartogram is converted into an orthogonal net on the surface  $\Sigma$ .

*The system (A) of azimuthal curves and the system (S) of scale curves are identical if, and only if, the cartogram is an equiareal map followed by a magnification.*

For the general cartograms not of the above type, there are  $5 \infty^1$  curves which are simultaneously azimuthal and scale curves. These are (a) the  $2 \infty^1$  minimal lines on the plane  $\pi$ , (b) the  $2 \infty^1$  minimal curves on the surface  $\Sigma$ , and (c) the  $\infty^1$  curves  $H(x, y) = \text{const.}$

Through a fixed lineal element of the plane, there pass, in general, a single azimuthal curve and a single scale curve. Let  $K_A$  be the curvature of the azimuthal curve, and let  $K_S$  be that of the scale curve, both of which are calculated at this given lineal element.

*By (A) and (S), it is found that the ratio  $K_A/K_S$  is determined by the formula*

$$1 - \frac{K_A}{K_S} = \frac{(H_x + H_y y')(E + 2Fy' + Gy'^2)}{H[E_x + (E_y + 2F_x)y' + (2F_y + G_x)y'^2 + G_y y'^3]}. \quad (6)$$

*Thus the ratio  $K_A/K_S$  is a rational function of third degree in  $y'$  with coefficients functions of  $(x, y)$ .*

In special cases, this ratio  $K_A/K_S$  may be a rational function of second or first degree in  $y'$  with coefficients functions of  $(x, y)$ . It may be even independent of the slope  $y'$ , and thus depend only on the position of the point.

5. *Cartograms for Which the Azimuthal Curves Are Straight.*—In our previous work, we found a new class of surfaces for which there exist cartograms whose  $\infty^2$  scale curves are all straight lines. Concerning the analogous problem for azimuthal straight lines, it is found that any arbitrary surface  $\Sigma$  may be mapped upon a plane  $\pi$  such that the azimuthal curves are all straight lines. We prove the following result.

*If a cartogram of an arbitrary surface  $\Sigma$  is such that the azimuthal curves coincide with the totality of  $\infty^2$  straight lines, then it is a conformal representation of  $\Sigma$  upon the plane  $\pi$ , followed by an affine (not a motion) transformation in  $\pi$ .*

For if the system (A) of  $\infty^2$  azimuthal curves consists of straight lines only, it may be shown that

$$\frac{E}{H} = a_0 y^2 + 2a_1 y + a_2, \quad \frac{F}{H} = -a_0 xy - a_1 x + b_1 y + c_2, \quad \frac{G}{H} = a_0 x^2 - 2b_1 x + b_2. \quad (7)$$

Substitute these into the condition:  $H^2 = EG - F^2 > 0$ . We obtain the relations

$$a_2 b_2 - c_2^2 = 1, \quad a_0 a_2 - a_1^2 = a_0 c_2 - a_1 b_1 = a_0 b_2 - b_1^2 = a_1 c_2 - a_2 b_1 = a_1 b_2 - b_1 c_2 = 0. \quad (8)$$

From these, we deduce that  $a_0 = a_1 = b_1 = 0$ .

Upon dropping the subscripts, it follows from (7) and the preceding conditions that the linear element (1) must be written in the form

$$dS^2 = H(adx^2 + 2cdxdy + bdy^2), \quad (9)$$

where  $(a, b, c)$  are constants such that  $ab - c^2 = 1$ , and  $H$  is a positive function of  $(x, y)$ .

The preceding remarks complete the proof of the above italicized statement.

*If in a cartogram, the azimuthal curves and the scale curves all coincide with the totality of  $\infty^2$  straight lines, then the surface  $\Sigma$  is developable and the transformation is an unrolling of  $\Sigma$  upon the plane  $\pi$  followed by an affinity in  $\pi$ .*

This is obtained as a corollary of the work outlined above. For upon imposing the condition that the scale curves of the cartogram defined by (9) be all straight lines, we find that the function  $H$  is a non-zero positive constant.

The result concerning straight azimuths is strikingly different from the corresponding theorem about straight scales. Any surface  $\Sigma$  can be mapped upon the plane  $\pi$  with straight azimuths but only a very special class of surfaces  $\Sigma$  can be represented upon the plane  $\pi$  with straight scales.

\* Presented to the American Mathematical Society, April, 1946. The following papers are all by Kasner and De Cicco.

<sup>1</sup> "Scale Curves in Cartography," *Science*, 98, 324-325 (1943), and *Science News Letter*, March 25, 1944.

<sup>2</sup> "Scale Curves in Conformal Maps," these PROCEEDINGS, 30, 162-164 (1944). "Geometry of Scale Curves in Conformal Maps," *Am. Jour. Math.*, 67, 157-166 (1945). "Conformal Maps with Isothermal Systems of Scale Curves, *Ibid.*, 68, 137-166 (1946).

<sup>3</sup> "Scale Curves in General Cartography," these PROCEEDINGS, 30, 211-215 (1944). See *Am. Jour. Math.*, 68, 66-76 (1946). *Scale Curves and Cartograms*, Boletino del Instituto de Matematicas del Universidad de Litoral, Rosario, Argentina (1946). One of our results on the converse of Ptolemy's theorem has been given by Hilton, 1928.



## A GENERALIZATION OF THE WIENER-HOPF INTEGRAL EQUATION\*

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*I. Introduction.*—In this note we outline some results which we have obtained by generalizing the Wiener-Hopf integral equation.<sup>1</sup> This integral equation is of the form

$$f(x) = \lambda \int_0^\infty K(x-y)f(y)dy \quad x > 0 \quad (1.1)$$

where  $K(x)$  is a known function. If the kernel  $K(x)$  possesses a bilateral Fourier transform

$$\int_{-\infty}^{\infty} e^{-i\omega x} K(x) dx$$

which exists in some strip in the complex  $\omega$  plane  $\beta < \Im m \omega < \beta'$ , one can obtain the so-called "fundamental solutions"<sup>2</sup> of equation (1.1) with the aid of certain regularity properties of the Fourier transform. We generalize (1.1) in a twofold fashion. First, a method is given which will enable one to write a formal solution to the integral equation

$$f(x) = \lambda \int_0^\infty N(x+y)f(y)dy \quad x > 0 \quad (1.2)$$

where now  $N(x)$  is integrable in any interval which excludes the origin,  $O(e^{-x})$  as  $x \rightarrow \infty$  and  $O(1/x)$  as  $x \rightarrow 0$ . The plus sign in the kernel now changes the entire mode of attack. While Fourier transform methods still play a dominant rôle in our work, we cannot solve the equation (1.2) in one fell swoop as we do equation (1.1). Instead we find it necessary to take advantage of the special assumptions which we impose upon  $N(x)$  and decompose equation (1.2) into an infinite sequence of bilateral falting equations, each one of which depends on the solution of the previous one. These we can solve step by step and our final answer appears as an infinite series of integral operators acting on a known function which has been determined in the course of our work. Thus a formal solution can be given for the above-described class of integral equations and this solution depends only upon the operation of integration. The decomposition is in some respects reminiscent of the method of successive approximations employed in the solution of non-homogeneous linear integral equations.

In the second place, under special conditions which we shall discuss elsewhere, the class of integral equations

$$\int_0^\infty N_1(x-y)f(y)dy + \int_0^\infty N_2(x+y)f(y)dy = \lambda f(x) \quad x > 0 \quad (1.3)$$



may be reduced to an integral equation of the form (1.2). Here  $N_1(x)$  and  $N_2(x)$  are given functions of  $x$  with special growth properties at infinity and are otherwise integrable for infinite  $x$ .

*II. Formal Solution of Equation (1.2).*—Specifically we are concerned with finding those solutions of the equation (1.2) which are of the order  $x^\alpha$ ,  $-1 < \alpha < 0$  for  $x \rightarrow 0$ .  $\lambda$  is a parameter which takes on real values between 0 and  $1/\pi$  if we are to obtain real solutions which have the above-described order at the origin. Since  $N(x)$  is asymptotic to  $e^{-x}$  as  $x \rightarrow \infty$ , we obtain a solution of (1.2) which is of the same order as  $x \rightarrow \infty$ . We show that  $\alpha$  may be determined from a characteristic equation, which is of course expressed in terms of  $\lambda$ .

Our technique is now the following. Since  $f(x)$  approaches zero exponentially, as  $x$  becomes infinite and since  $N(x)$  is singular as  $x$  approaches zero, we take out the dominant part of the solution by decomposing equation (1.2) as follows. We rewrite equation (1.2) as the following system of integral equations

$$f_0(x) = \lambda \int_0^\infty \frac{f_0(y)}{x+y} dy \quad (2.1)$$

$$f_n(x) = \lambda \int_0^\infty \frac{f_n(y) dy}{x+y} + \lambda \int_0^\infty \left[ N(x+y) - \frac{1}{x+y} \right] f_{n-1}(y) dy; \\ n = 1, 2, \dots \quad (2.2)$$

where

$$f(x) = \sum_{n=0}^\infty f_n(x).$$

Equation (2.1) has been studied extensively in the literature and is known possess the solutions  $x^\alpha$ ,  $x^{-1-\alpha}$ , where  $\sin \alpha\pi = -\lambda\pi$ . It has been further shown by Hardy and Titchmarsh, that the only two linearly distinct solutions of (2.1) are of this form.

If we put  $x = e^t$ ,  $y = e^s$  equations (2.2) assume the form of bilateral faltung equations, that is

$$f_n(e^t)e^{t/2} = \lambda \int_{-\infty}^\infty \frac{f_n(e^s)e^{s/2} ds}{e^{(t-s)/2} + e^{(s-t)/2}} + \lambda e^{t/2} q_n(e^t); \quad n = 1, 2, \dots \quad (2.3)$$

where

$$q_n(x) = \int_0^\infty \left[ N(x+y) - \frac{1}{x+y} \right] f_{n-1}(y) dy.$$

Equations (2.3) can be solved with the bilateral Fourier transform theorem in the complex domain. The solution can be exhibited explicitly with the

aid of an integral operator which we do not write out here explicitly, but denote by  $P$ , that is,

$$f_n(x) = \lambda q_n(x) + Pq_n \quad (2.4)$$

where now it is understood that

$$Pq_n = \int_0^\infty P(x, t)q_n(t)dt$$

and  $P(x, t)$  is a known function of  $x$  and  $t$ . If we denote the integral operations

$$\int_0^\infty N(x + y)f_n(y)dy \quad \text{by } Nf_n$$

and

$$\int_0^\infty \frac{f_n(y)dy}{x + y} \quad \text{by } Lf_n$$

we observe that the equations (2.2) can be rewritten as

$$f_n(x) = \lambda Lf_n + \lambda Nf_{n-1} - \lambda Lf_{n-1} \quad (2.5)$$

and  $q_n(x)$  as

$$q_n(x) = Nf_{n-1} - Lf_{n-1}.$$

We then get immediately,

$$f_n(x) = \lambda(1 + P)(\lambda NP + \lambda N - P)^{n-1}q_1$$

where the exponent  $n - 1$  indicates that the operation within the parentheses is to be applied  $n - 1$  times and furthermore the operators  $N$  and  $P$  do not commute. The final formal solution is then

$$f(x) = f_0(x) + \sum_{n=1}^{\infty} \lambda(1 + P)(\lambda NP + \lambda N - P)^{n-1}q_1$$

and this indeed satisfies the integral equation (1.2).

*III. An Example.*—If we now apply the previously described technique to the integral equation

$$f(x) = \lambda \int_0^\infty \frac{e^{-(x+y)}f(y)}{x + y} dy$$

we find that  $f(x)$  is  $K_{\alpha+1/2}(x)/\sqrt{x}$ , where now  $\alpha$  is defined by the constraining equation  $\lambda\pi = -\sin \pi\alpha$ ,  $0 < \lambda\pi < 1$  and  $-1 < \alpha < 0$ . The function  $K_{\alpha+1/2}(x)$  is a modified Bessel function of the second kind which is invariant under the substitution of replacing  $\alpha$  by  $-\alpha - 1$ . It is clear that  $f(x)$  is of the order  $x^\alpha$  or  $x^{-\alpha-1}$  for small  $x$ , depending upon which term dominates. For  $x$  large and positive,  $f(x)$  is of the order  $e^{-x}$ .

A word of description as to how we succeeded in obtaining the solution in closed form is in order. The procedure indicated in Section II leads to an infinite expansion of iterated integral operators. For this particular example, this expansion can be shown to be the solution of a Wiener-Hopf integral equation, which of course can be solved explicitly. There is much hope that the Wiener-Hopf technique will play an important rôle in the solution of integral equations of the form (1.2) when the exponential character of the kernel  $N(x + y)$  is exhibited explicitly as it is in the above example.

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<sup>1</sup> Paley and Wiener, "Fourier Transforms in the Complex Domain," *Colloq. Pub. Am. Math. Soc.*, Chapter 4, 1934.

<sup>2</sup> Paley and Wiener, loc. cit., Chapter 4.

## ON CLASSES OF DIOPHANTINE EQUATIONS OF HIGHER DEGREES WHICH HAVE NO SOLUTIONS

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This paper is concerned with the isolation of certain classes of Diophantine equations which have no solutions or no non-zero solutions, using the theory of congruences. Let  $f(x_1, x_2, \dots, x_s)$  and  $g(y_1, y_2, \dots, y_t)$  be polynomials in  $x_1, x_2, \dots, x_s$  and  $y_1, y_2, \dots, y_t$  with integral coefficients. Then if  $m$  is an integer and

$$f(x_1, x_2, \dots, x_s) \equiv 0 \pmod{m} \quad (1)$$

has no solutions in  $x_1, x_2, \dots, x_s$ , then it is obvious that the equation

$$f(x_1, x_2, \dots, x_s) + mg(y_1, y_2, \dots, y_t) = 0 \quad (2)$$

has no solution in integers  $x_1, x_2, \dots, x_s; y_1, y_2, \dots, y_t$ . However, if  $f(x_1, x_2, \dots, x_s)$  is homogeneous, then the congruence (1) has the solution  $x_1 = x_2 = \dots = x_s = 0$ , so that this principle cannot be employed immediately for homogeneous equations. Also it may be shown by the use of congruences that the equation

$$v^3 - u^4 = 10$$

has no solutions, but its relation to an equation of the form (2) is not ob-

vious. Here we shall establish types of congruences which have no solutions or only zero solutions and apply these results directly to deriving Diophantine equations with no solutions.

We shall first illustrate the principal method by examining the special equation, with  $x, y, z$  not all zero,

$$x^4 + y^4 - 3z^4 = 0. \quad (3)$$

Taking first the case in which 5 is not a divisor of  $x, y$  or  $z$ , we have the congruence

$$x^4 + y^4 - 3z^4 \equiv 0 \pmod{5}, \quad (4)$$

which by Fermat's minor theorem yields the impossibility  $1 + 1 - 3 = -1 \equiv 0 \pmod{5}$ . If, however, 5 divides  $x, y$  or  $z$ , and since  $xyz$  are not all 0, we may divide the equation (3) by  $5^a$ , where  $5^a$  is the greatest power of 5 that divides all the numbers  $x, y$  and  $z$ . This results in an equation of the type

$$x_1^4 + y_1^4 - 3z_1^4 = 0$$

in which not more than one of the numbers  $x_1, y_1$  and  $z_1$  has the factor 5. This is easily shown to be impossible, hence (3) is impossible. We may secure other equations having no non-zero solutions by multiplying (3) by any number relatively prime to 5 and adding any multiples of 5 to the coefficients. Or, again, we might consider the equation

$$x^8 + 2y^8 + 4z^8 - 8u^8 = 0 \quad (5)$$

with  $x, y, z$  and  $u$  not all  $= 0$ . As before, division by  $17^a$ , the highest power of 17 common to all four terms, gives an equation of the type (5) in which two, at most, of the terms are multiples of 17. Since  $8 = \frac{17-1}{2}$  and

$$x_2^8 \equiv -2y_2^8 - 4z_2^8 + 8u_2^8 \pmod{17},$$

it follows that

$$b \equiv -2c - 4d + 8e \pmod{17},$$

where  $b, c, d$  and  $e$  range over the values 1,  $-1$  and 0 independently except that not more than 2 of them can  $= 0$ . If  $b \neq 0$ , then we have the impossibility of  $\equiv 1$  congruent to the sum, which is  $< 17$  but  $> -17$ , of three even numbers, while if  $b = 0$ , we note in turn, for the cases  $e \neq 0$  and  $e = 0$ , that  $|8e| > |-2c| + |-4d|$  and  $|-4d| > |-2c|$ , so that the congruence is impossible in any event. As before, multiplication of (5) by numbers relatively prime to 17 and adding any multiples of 17 to the coefficients will give new Diophantine equations having no non-zero solutions.

To obtain general results we extend a method due to H. H. Mitchell<sup>1</sup> for attacking the problem of finding the primes  $p$  such that

$$x^m + y^m + 1 \equiv 0 \pmod{p},$$

with  $x$  and  $y$  integers,  $xy \not\equiv 0 \pmod{p}$ , and  $p = 1 + mc$  where  $m$  is a prime. Consider the congruence

$$\begin{aligned} k + a_1 x_1^m + a_2 x_2^m + \dots + a_s x_s^m &\equiv 0 \pmod{p}, \\ a_1 a_2 \dots a_s x_1 x_2 \dots x_s &\not\equiv 0 \pmod{p}, \end{aligned} \quad (6)$$

with  $m$  not necessarily prime, but with all the letters representing integers. Using the method described in a previous paper,<sup>1</sup> it follows that if  $\alpha$  is a primitive  $c^{\text{th}}$  root of unity we shall have for some  $r$ 's

$$k + a_1 \alpha^{r_1} + a_2 \alpha^{r_2} + \dots + a_s \alpha^{r_s} \equiv 0 \pmod{\mathfrak{p}} \quad (7)$$

where  $\mathfrak{p}$  is a prime ideal divisor of  $p$  in the algebraic field defined by  $e^{2\pi i/c}$ . Let  $N(\omega)$  be the norm of  $\omega$ . Then since the norm is rational we have

$$N(k + a_1 \alpha^{r_1} + \dots + a_s \alpha^{r_s}) \equiv 0 \pmod{p}. \quad (8)$$

Now

$$|k + a_1 \alpha^{r_1} + \dots + a_s \alpha^{r_s}| \leq |k| + |a_1 \alpha^{r_1}| + \dots + |a_s \alpha^{r_s}| \leq |k| + |a_1| + |a_2| + \dots + |a_s|$$

since the absolute value of  $\alpha^r$  is unity; and the same result holds for any of the conjugates of this number, hence

$$N(k + a_1 \alpha^{r_1} + \dots + a_s \alpha^{r_s}) \leq (|k| + |a_1| + |a_2| + \dots + |a_s|)^{\varphi(c)}$$

Therefore, unless one of the factors in the left-hand member of (8) is zero, we must have

$$(|k| + |a_1| + |a_2| + \dots + |a_s|)^{\varphi(c)} \leq p,$$

but this is independent of  $m$ , so we can select  $m$  so large that

$$(|k| + |a_1| + |a_2| + \dots + |a_s|)^{\varphi(c)} < p, \quad (9)$$

whence the

**THEOREM I.** *If  $c$  is a given integer  $> 0$ , and  $p$  is a prime such that  $p = 1 + mc$  with  $m$  an integer, and  $(|k| + |a_1| + |a_2| + \dots + |a_s|)^{\varphi(c)} < p$ , and  $k + a_1 \alpha^{r_1} + a_2 \alpha^{r_2} + \dots + a_s \alpha^{r_s} \neq 0$  for  $\alpha = e^{2\pi i/c}$  and  $r_1, r_2, \dots, r_s$  any integers, then  $k + a_1 x_1^m + a_2 x_2^m + \dots + a_s x_s^m \equiv 0 \pmod{p}$  has no solutions  $x_1, x_2, \dots, x_s$  if  $x_1 x_2 \dots x_s \not\equiv 0 \pmod{p}$  and  $a_1 \dots a_s \neq 0$ .*

Now suppose we consider the equation

$$k + a_1 y_1^m + a_2 y_2^m + \dots + a_s y_s^m = 0, \quad (10)$$

and use the prime  $p$  as defined in Theorem I with the conditions given on  $p$

and the  $a$ 's. By Theorem I, (10) has no solutions in integers  $y_1, y_2, \dots, y_s$  prime to  $p$ . Hence, if it has any solutions some of the  $y$ 's are divisible by  $p$ . We may choose our notation so that those not divisible by  $p$  are  $y_1, y_2, \dots, y_t$ ;  $0 < t < s$ . Hence if (10) is satisfied it gives the congruence

$$k + a_1 y_1^m + a_2 y_2^m + \dots + a_t y_t^m \equiv 0 \pmod{p},$$

$$y_1 y_2 \dots y_t \not\equiv 0 \pmod{p}. \quad (11)$$

Now apply the criterion of Theorem I to this congruence, and we see that (11) has no solutions prime to  $p$  since the condition (9) is satisfied, provided, however, that  $k + a_1 \alpha_1^{e_1} + a_2 \alpha_2^{e_2} + \dots + a_t \alpha_t^{e_t} \not\equiv 0$ , where  $e_1, e_2, \dots, e_t$  are arbitrary integers. This gives the

**THEOREM II.** *If  $c$  is a given integer  $> 0$ , and it is possible to find an integer  $m$  and a prime  $p$  such that  $p = 1 + mc$ , also integers  $k, a_1, a_2, \dots, a_s$  so that  $a_1 a_2 \dots a_s \not\equiv 0$  and*

$$(|k| + |a_1| + |a_2| + \dots + |a_s|)^{p(c)} < p$$

*and none of the expressions*

$$k + a_1 \alpha_1 + a_2 \alpha_2 + \dots + a_s \alpha_s \quad (12)$$

*is zero where any  $j$  of the  $\alpha$ 's are zero,  $0 \leq j < s$ , and the non-zero  $\alpha$ 's are any roots of  $x^c = 1$  then the equation*

$$k + a_1 y_1^m + a_2 y_2^m + \dots + a_s y_s^m = 0$$

*is impossible in integers  $y_1, y_2, \dots, y_s$  except in the case when  $k = 0$ , and then  $y_1 = y_2 = \dots = y_s = 0$ .*

Now assume that  $k = 0$  and  $c$  is a prime, say  $q$ . We examine the condition (12) and assume that  $i$  of the  $\alpha$ 's differ from zero,  $0 < i \leq s$ . Then these  $\alpha$ 's are  $q$ th roots of unity and the primitive roots satisfy

$$\frac{x^q - 1}{x - 1} = x^{q-1} + x^{q-2} + \dots + 1 = 0. \quad (13)$$

Assume that  $s \leq q - 2$ . If  $\alpha$  is a primitive root of  $x^q = 1$ , all the roots are given by  $\alpha, \alpha^2, \dots, \alpha^q$ . Now consider the  $\alpha$ 's which appear in (12). If none equals  $\alpha^q - 1$ , then (12) is not zero since the left-hand member of (13) is irreducible in the rational field. Say that  $d$  of them equal  $\alpha^q - 1$  and call these  $\alpha_1, \alpha_2, \dots, \alpha_d$  with  $0 < d \leq i$ , where  $i$  of the  $\alpha$ 's differ from zero as noted above. Then using

$$\alpha^q - 1 = -\alpha^{q-2} - \alpha^{q-3} - \dots - 1,$$

the expression (12) becomes

$$-(a_1 + a_2 + \dots + a_d)(\alpha^{q-2} + \alpha^{q-3} + \dots + 1) + a_{d+1}\alpha_{d+1} + \dots + a_s\alpha_s. \quad (14)$$



Consider the terms

$$a_{d+1}\alpha_{d+1} + \dots + a_s\alpha_s.$$

In number they are  $< q - 2$  since  $s \leq q - 2$ . Hence if we write (14) in the form

$$h_0 + h_1\alpha + h_2\alpha^2 + \dots + h_{q-2}\alpha^{q-2} \quad (15)$$

in order for it to be zero each  $h$  is zero. But unless  $(a_1 + a_2 + \dots + a_d)$  in (14) is zero then there is a power of  $\alpha$  appearing in (15) whose coefficient  $-(a_1 + a_2 + \dots + a_d)$  is not zero and which is not in the set

$$\alpha_{d+1}, \dots, \alpha_s$$

and this is impossible. Hence

$$a_1 + a_2 + \dots + a_d = 0, \quad (16)$$

and this gives from (14) and (15)

$$a_{d+1}\alpha_{d+1} + \dots + a_s\alpha_s = 0$$

and this is impossible unless  $d = i$ . Hence (16) holds for any  $d$  of the  $a$ 's  $0 < d \leq s$ . Whence the

**THEOREM III.** *If  $c$  is a prime and  $m$  is an integer such that  $p = 1 + mc$  with  $p$  prime, then*

$$a_1x_1^m + a_2x_2^m + \dots + a_sx_s^m = 0 \quad (17)$$

*has only the solution  $x_1 = x_2 = \dots = x_s = 0$  provided that  $s \leq c - 2$ ; the sum of no  $n$  of the  $a$ 's is zero,  $0 < n \leq s$ ; and*

$$(|a_1| + |a_2| + \dots + |a_s|)^{p(c)} < p.$$

*The condition that the sum of no  $n$  of the  $a$ 's is zero is necessary since if*

$$a_1 + a_2 + \dots + a_j = 0$$

*then (17) is satisfied with*

$$\begin{aligned} x_1 &= x_2 = \dots = x_j = 1 \\ x_{j+1} &= x_{j+2} = \dots = x_s = 0. \end{aligned}$$

For the least non-trivial value of  $c$ , viz.,  $c = 5$ , the theorem shows, for example, that

$$x^m + y^m - 3z^m = 0 \quad (18)$$

is impossible for any  $m$  such that  $p = 1 + 5m$  and  $p > 5^4$  unless  $x = y = z = 0$ . The smallest such  $p$  is 631 giving  $m = 126$ , so that (18) has no non-zero solutions for  $m = 126$ , and also

$$ax^{126} + by^{126} + cz^{126} = 0$$

is impossible for any  $a$ ,  $b$  and  $c$  such that there exists a  $k$  satisfying  $ka \equiv kb \equiv 1 \pmod{631}$  and  $kc \equiv -3 \pmod{631}$ .

<sup>1</sup> Vandiver, H. S., these PROCEEDINGS, 30, 368-370 (1944).

## THE TRANSFORMATION OF DYNAMICAL SYSTEMS OF TWO DEGREES OF FREEDOM

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1. *Introduction.*—Let  $D$  and  $E$  be dynamical systems of  $n$  degrees of freedom which are referred to the same set of generalized coördinates  $x^1, \dots, x^n$ . We represent these systems by the Lagrangian equations

$$D: \frac{d^2x^\alpha}{dt^2} + \Gamma_{\mu\nu}^\alpha \frac{dx^\mu}{dt} \frac{dx^\nu}{dt} = Q^\alpha, \quad (1.1)$$

$$E: \frac{d^2x^\alpha}{d\tau^2} + \Lambda_{\mu\nu}^\alpha \frac{dx^\mu}{d\tau} \frac{dx^\nu}{d\tau} = R^\alpha. \quad (1.2)$$

It is assumed that the generalized force vectors  $Q$  and  $R$  depend on the coördinates  $x$  alone and that neither vector vanishes in the region under consideration.<sup>1</sup> The kinetic energies of  $D$  and  $E$  are assumed to have the form

$$\frac{1}{2}g_{\alpha\beta}(x)\frac{dx^\alpha}{dt}\frac{dx^\beta}{dt} \text{ and } \frac{1}{2}h_{\alpha\beta}(x)\frac{dx^\alpha}{d\tau}\frac{dx^\beta}{d\tau}.$$

The above quantities  $\Gamma_{\mu\nu}^\alpha$  and  $\Lambda_{\mu\nu}^\alpha$  are Christoffel symbols derived from the coefficients  $g_{\alpha\beta}$  and  $h_{\alpha\beta}$  of these forms, respectively.

In a paper previously written<sup>2</sup> we have given necessary and sufficient conditions for all trajectories of  $E$  to be trajectories of  $D$  under the assumption that these systems involved more than two degrees of freedom; in particular this led to conditions which were both necessary and sufficient for  $D$  and  $E$  to have identical trajectories.

In this note we derive the formulas (§ 2) which enable us to say that all theorems obtained in the above paper are likewise valid in the case  $n = 2$ . However, in the exceptional case of two degrees of freedom results can be stated which are preferable to those previously obtained for systems of more than two degrees of freedom. Conditions are given in § 4 for the

identity of all trajectories of  $D$  and  $E$  for the general case of systems of two degrees of freedom and in § 5 for the identity of the trajectories of  $D$  and  $E$  when these systems are conservative.

2. *The Fundamental Algebraic Relations.*—Assuming all trajectories of  $E$  to be trajectories of  $D$  and that the parameter transformations  $t \rightarrow \tau$  and  $\tau \rightarrow t$  are of class  $C^2$ , we have

$$\left( \Phi_{\mu\nu}^{\alpha} \frac{dx^{\beta}}{d\tau} - \Phi_{\mu\nu}^{\beta} \frac{dx^{\alpha}}{d\tau} \right) \frac{dx^{\mu}}{d\tau} \frac{dx^{\nu}}{d\tau} = \left( R^{\alpha} \frac{dx^{\beta}}{d\tau} - R^{\beta} \frac{dx^{\alpha}}{d\tau} \right) - \left( Q^{\alpha} \frac{dx^{\beta}}{d\tau} - Q^{\beta} \frac{dx^{\alpha}}{d\tau} \right) \left( \frac{dt}{d\tau} \right)^2, \quad (2.1)$$

where,

$$\Phi_{\mu\nu}^{\alpha} = \Lambda_{\mu\nu}^{\alpha} - \Gamma_{\mu\nu}^{\alpha},$$

along any trajectory of  $E$ . See equation (2.3) of the previous paper<sup>2</sup> (hereafter referred to by the initials T.E.D.). Suppose initially that  $dx^{\alpha}/d\tau = kQ^{\alpha}$ , where  $k$  is a constant. Then from (2.1) we obtain

$$k^2(\Phi_{\mu\nu}^{\alpha} Q^{\beta} - \Phi_{\mu\nu}^{\beta} Q^{\alpha}) Q^{\mu} Q^{\nu} = k(R^{\alpha} Q^{\beta} - R^{\beta} Q^{\alpha}).$$

Since the constant  $k$  is arbitrary these relations imply

$$R^{\alpha} Q^{\beta} - R^{\beta} Q^{\alpha} = 0, \quad (\Phi_{\mu\nu}^{\alpha} Q^{\mu} Q^{\nu}) Q^{\beta} - (\Phi_{\mu\nu}^{\beta} Q^{\mu} Q^{\nu}) Q^{\alpha} = 0.$$

Hence it follows that  $R^{\alpha}$  and  $\Phi_{\mu\nu}^{\alpha} Q^{\mu} Q^{\nu}$  are proportional to the  $Q^{\alpha}$ , i.e.,

$$R^{\alpha} = \psi Q^{\alpha}, \quad \Phi_{\mu\nu}^{\alpha} Q^{\mu} Q^{\nu} = L Q^{\alpha}, \quad (2.2)$$

where the proportionality factors  $\psi$  and  $L$  may depend on the coördinates  $x$ .

In T.E.D. we showed that the quantities  $\Phi_{\mu\nu}^{\alpha}$  must be of the form

$$\Phi_{\mu\nu}^{\alpha} = \delta_{\mu}^{\alpha} \phi_{\nu} + \delta_{\nu}^{\alpha} \phi_{\mu} + A_{\mu\nu} Q^{\alpha}; \quad (2.3)$$

we now show that these relations likewise hold for the case  $n = 2$ . Considering that the  $\Phi$ 's and  $Q$ 's are known, the quantities  $\phi$ , and the symmetric quantities  $A_{\mu\nu}$  are determined from (2.3) in accordance with the following equations

$$\left. \begin{aligned} \Phi_{22}^1 &= A_{22} Q^1 && \text{(determines } A_{22}) \\ \Phi_{11}^2 &= A_{11} Q^2 && \text{(determines } A_{11}) \\ \Phi_{11}^1 &= 2\phi_1 + A_{11} Q^1 && \text{(determines } \phi_1) \\ \Phi_{22}^2 &= 2\phi_2 + A_{22} Q^2 && \text{(determines } \phi_2) \\ \Phi_{12}^1 &= \phi_2 + A_{12} Q^1 && \text{(determines } A_{12}). \end{aligned} \right\} \quad (2.4)$$

In making these determinations it is supposed that neither of the components  $Q^1$  and  $Q^2$  vanishes; this condition can be realized in the neighbor-

hood of any point  $P$  by a proper choice of coördinates since we have assumed that the vector  $Q$  does not vanish.

In view of (2.4) all equations (2.3) hold with the possible exception of the equation corresponding to  $\alpha = 2, \mu = 1, \nu = 2$ . Now put

$$\Phi_{12}^2 = \phi_1 + A_{12}Q^2 + K; \quad (2.5)$$

the remaining equation (2.3) will then be satisfied if we can show that  $K = 0$ . But substituting the values of the  $\Phi$ 's from (2.4) and (2.5) into the second set of equations (2.2) and eliminating the factor  $L$  between these two equations we are led to the relation  $K(Q^1)^2Q^2 = 0$ . Hence,  $K = 0$  and (2.3) is necessarily satisfied.

On account of (2.3) and the relation  $R^\alpha = \psi Q^\alpha$  it is immediately seen by recourse to T.E.D. that the general discussion and all results obtained in the previous paper are valid for systems of two degrees of freedom.

3. *Two-Dimensional Riemann Spaces with Corresponding Geodesics.*—Let

$$\lambda_{\alpha\beta} \frac{dx^\alpha}{dt} \frac{dx^\beta}{dt} = \text{const.} \quad (3.1)$$

be a first integral of the differential equations of the geodesics of a Riemann space  $R$  with metric defined by  $g_{\alpha\beta}$ . It is well known that the necessary and sufficient conditions for the existence of this integral are that

$$\lambda_{\alpha\beta, \gamma} + \lambda_{\beta\gamma, \alpha} + \lambda_{\gamma\alpha, \beta} = 0, \quad (3.2)$$

where the "comma" denotes covariant differentiation based on the Christoffel symbols  $\Gamma_{\mu\nu}^\alpha$  determined by the  $g_{\alpha\beta}$ . For the two-dimensional case the relations (3.2), when written in full, become

$$\lambda_{11, 2} + 2\lambda_{12, 1} = 0, \quad \lambda_{22, 1} + 2\lambda_{12, 2} = 0, \quad \lambda_{11, 1} = \lambda_{22, 2} = 0. \quad (3.3)$$

Now determine functions  $\mu_1$  and  $\mu_2$  as solutions of the two equations

$$\left. \begin{aligned} \lambda_{11}\mu_2 - \lambda_{12}\mu_1 &= \lambda_{11, 2} \\ -\lambda_{12}\mu_2 + \lambda_{22}\mu_1 &= \lambda_{22, 1} \end{aligned} \right\} \quad (3.4)$$

Assuming  $\det. |\lambda_{\alpha\beta}| \neq 0$ , these equations have a unique solution  $\mu_1$  and  $\mu_2$ . Hence

$$\left. \begin{aligned} \lambda_{12, 1} &= -\frac{1}{2}\lambda_{11}\mu_2 + \frac{1}{2}\lambda_{12}\mu_1 \\ \lambda_{12, 2} &= \frac{1}{2}\lambda_{12}\mu_2 - \frac{1}{2}\lambda_{22}\mu_1 \end{aligned} \right\} \quad (3.5)$$

in consequence of the first two equations (3.3). Equations (3.4) and (3.5) can be written in the combined form

$$\lambda_{\alpha\beta, \gamma} = \lambda_{\alpha\beta}\mu_\gamma - \frac{1}{2}\lambda_{\alpha\gamma}\mu_\beta - \frac{1}{2}\lambda_{\beta\gamma}\mu_\alpha. \quad (3.6)$$

All conditions (3.3) are now seen to be satisfied by the  $\lambda_{\alpha\beta, \gamma}$  as given by (3.6) which is thus an allowable expression for these quantities.

From (3.6) we can deduce  $\mu_\alpha = (\log \lambda/g)_{,\alpha}$  where  $\lambda = \det. | \lambda_{\alpha\beta} |$  and  $g = \det. | g_{\alpha\beta} |$ . Hence  $\mu_\alpha$  is the gradient of a scalar  $\mu$ . Putting  $\mu = \log \nu$ , we have  $\nu = c\lambda/g$ , where  $c$  is a constant. Introducing the function  $\nu$  into (3.6) this equation can be given the form

$$h_{\alpha\beta,\gamma} = 2h_{\alpha\beta}\eta_{,\gamma} + h_{\alpha\gamma}\eta_{,\beta} + h_{\beta\gamma}\eta_{,\alpha} \quad (3.7)$$

where

$$h_{\alpha\beta} = \frac{\lambda_{\alpha\beta}}{\nu^2} \text{ and } \eta = -\frac{1}{2} \log \nu.$$

But (3.7) is the condition<sup>3</sup> that  $h_{\alpha\beta}$  defines the metric of a Riemann space  $R'$  having the same geodesics as  $R$ .<sup>4</sup>

Conversely if a Riemann space  $R'$ , with metric defined by  $h_{\alpha\beta}$ , has the same geodesics as  $R$ , equations of the form (3.7) hold. By retracing our steps we are thus led to the existence of a quadratic first integral (3.1) of the differential equations of the geodesics of  $R$ . The result proved can be stated in the following terms: *The totality of Riemann spaces  $R'$ , whose geodesics are the same as the geodesics of a given Riemann space  $R$ , is derivable, as above, from the set of all first integrals (3.1) of the differential equations of the geodesics of  $R$ .*

Now let

$$g_{\mu\nu}^{(i)} \frac{dx^\mu}{dt} \frac{dx^\nu}{dt} = \text{const.}, \quad (i = 1, \dots, s), \quad (3.8)$$

be a basis<sup>5</sup> of quadratic first integrals of the differential equations of the geodesics of  $R$ . The above italicized result can now be embodied in the following theorem.

**THEOREM.** *The geodesics of the two-dimensional Riemann space  $R'$  will be the same as the geodesics of the two-dimensional Riemann space  $R$  if, and only if,*

$$h_{\alpha\beta} = \left( \frac{\det. | g_{\mu\nu} |}{\det. | c_h g_{\mu\nu}^{(h)} |} \right)^2 c_i g_{\alpha\beta}^{(i)}$$

where the  $g_{\mu\nu}^{(i)}$  are the coefficients in a basis of quadratic first integrals (3.8) of  $R$  and the  $c$ 's are constants.

If  $R$  and  $R'$  are Riemann spaces in the strict sense the forms  $g_{\alpha\beta}\xi^\alpha\xi^\beta$  and  $h_{\alpha\beta}\xi^\alpha\xi^\beta$  are positive definite; then the constants  $c_i$  must be such that the  $h_{\alpha\beta}$  in the above equations are the coefficients of a positive definite quadratic form.

**4. General Correspondence Theorem for Dynamical Systems.**—The theorem of § 3 combined with the italicized result at the end of § 7 in T.E.D. leads immediately to the following theorem.

The trajectories of the dynamical system  $E$  of two degrees of freedom will be the same as the trajectories of the system  $D$  of two degrees of freedom if, and only if,

$$h_{\alpha\beta} = \left( \frac{\det. | g_{\mu\nu} |}{\det. | c_k g_{\mu\nu}^{(k)} |} \right)^2 c_i g_{\alpha\beta}^{(i)}$$

$$R^\alpha = c^2 \left( \frac{\det. | c_k g_{\mu\nu}^{(k)} |}{\det. | g_{\mu\nu} |} \right)^2 Q^\alpha$$

where the  $g_{\mu\nu}^{(i)}$  are the coefficients in a basis of quadratic first integrals (3.8) of the differential equations of the geodesics of  $D$  and the  $c$ 's are constants.

5. *Correspondence of Conservative Systems.*—Suppose now that the systems  $D$  and  $E$  are conservative so that we have  $Q^\alpha = -g^{\alpha\sigma} V_{,\sigma}$  and  $R^\alpha = -h^{\alpha\sigma} W_{,\sigma}$  where the potentials  $V$  and  $W$  depend on the coördinates  $x$  alone. Also, let

$$\frac{1}{2} g_{\mu\nu}^{(i)} \frac{dx^\mu}{dt} \frac{dx^\nu}{dt} + V^{(i)} = \text{const.}, \quad (i = 1, \dots, s), \quad (5.1)$$

be a basis<sup>5</sup> of quadratic first integrals of energy type for the system  $D$ .

The requirement that the trajectories of  $D$  and  $E$  are the same necessitates the vanishing of the quantities  $A_{\mu\nu}$  in T.E.D. (see § 7 of T.E.D.). Hence the constants  $m_i = 0$  in § 8 of T.E.D. and the equations (8.10) and (8.14) of this paper lead to the same expression for the quantities  $h_{\alpha\beta}$  as in the italicized theorem in the preceding section although now the  $g_{\alpha\beta}^{(i)}$  are the coefficients of the basis of integrals (5.1).

The relation between the  $R^\alpha$  and  $Q^\alpha$  in the theorem of § 4 is necessarily satisfied when the trajectories of  $D$  and  $E$  are the same and this relation is now equivalent to the following

$$\begin{aligned} W_{,\beta} &= c^2 \left( \frac{\det. | c_k g_{\mu\nu}^{(k)} |}{\det. | g_{\mu\nu} |} \right)^2 h_{\alpha\beta} g^{\alpha\sigma} V_{,\sigma} \\ &= c^2 c_i g_{\alpha\beta}^{(i)} g^{\alpha\sigma} V_{,\sigma} \\ &= c^2 c_i V_{,\beta}^{(i)} = [c^2 c_i V^{(i)}]_{,\beta} \end{aligned}$$

where use has been made of the differential conditions for (5.1) to be a first integral of the system (1.1). Hence  $W = c^2 c_i V^{(i)} + \text{const.}$  Conversely this equation implies the relation between the vectors  $R$  and  $Q$  appearing in the theorem of § 4.

These results together with the fact that the relations in the theorem of § 4 are necessary and sufficient for the identity of the trajectories of  $D$  and  $E$  give us the following theorem. *The trajectories of the conservative system  $E$  of two degrees of freedom will be the same as the trajectories of the conservative system  $D$  of two degrees of freedom if, and only if,*



$$h_{\alpha\beta} = \left( \frac{\det. |g_{\mu\nu}|}{\det. |c_i g_{\mu\nu}^{(i)}|} \right)^2 c_i g_{\alpha\beta}^{(i)}$$

$$W = c^2 c_i V^{(i)} + d$$

where the  $g_{\mu\nu}^{(i)}$  and  $V^{(i)}$  are the coefficients in a basis of quadratic first integrals (5.1) of the system  $D$  and the  $c$ 's and  $d$  are constants.

The quadratic forms  $g_{\alpha\beta}\xi^\alpha\xi^\beta$  and  $h_{\alpha\beta}\xi^\alpha\xi^\beta$  are positive definite in the dynamical problem; hence it is understood implicitly in the above theorem and in the theorem in § 4 that the constants  $c_i$  are to be chosen so that the  $h_{\alpha\beta}$  are the coefficients of a positive definite quadratic form. Moreover, since it is assumed that the force vector  $Q$  does not vanish, the constant  $c$  appearing in each of these theorems must be different from zero.<sup>1</sup>

<sup>1</sup> It can be shown that if one of the vectors  $Q$  and  $R$  vanishes at a point the other vector must likewise vanish at that point if the trajectories of the two systems are the same.

<sup>2</sup> "On the Transformation of the Equations of Dynamics," to appear in the June issue of the *Journal of Mathematics and Physics* (1946). Referred to in the text by the initials T.E.D.

<sup>3</sup> See Eisenhart, L. P., *Riemannian Geometry*, Princeton, 1926, p. 133.

<sup>4</sup> The fact that  $\lambda_{\alpha\beta}/\nu^2$  defines the metric of a Riemann space having the same geodesics as  $R$  appears to be known since the result is stated as a problem on p. 335 of Whittaker, E. T., *Analytical Dynamics*, 4th ed., Dover Publications, 1944. However it is desirable to have the equations leading to this result in order to infer the theorem of § 3.

<sup>5</sup> Thomas, T. Y., "The Fundamental Theorem on Quadratic First Integrals," these PROCEEDINGS, 32, 10-15 (1946).

## THE COEFFICIENTS OF SCHLICHT FUNCTIONS, III

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For functions

$$f(z) = z + a_2 z^2 + a_3 z^3 + \dots + a_n z^n + \dots$$

which are regular and schlicht in  $|z| < 1$ , the determination of the regions of variability of the coefficients is a classical problem—the so-called coefficient problem for schlicht functions. Given the point  $(a_2, a_3, \dots, a_n)$  we say that

$$f(z) = z + b_2 z^2 + b_3 z^3 + \dots + b_n z^n + \dots$$

belongs to the point  $(a_2, a_3, \dots, a_n)$  if  $f(z)$  is regular and schlicht in  $|z| < 1$  and

$$b_2 = a_2, b_3 = a_3, \dots, b_n = a_n.$$

Conversely we say that the point  $(a_2, a_3, \dots, a_n)$  belongs to  $f(z)$ . The  $n$ th region of variability  $V_n$  lies in a Euclidean space of  $2n - 2$  real dimensions and is the set of points  $(a_2, a_3, \dots, a_n)$  each of which belongs to some  $f(z)$ . Only  $V_2$  has been determined heretofore, and it is the circle  $|a_2| \leq 2$ .

In this note we outline a method which gives  $V_n$  for general  $n$ . The boundary of  $V_3$  may be expressed by equations involving only elementary functions, but for  $n > 3$  the boundary of  $V_n$  is quite complicated and cannot be so expressed. Here, therefore, we shall write out the equations of the boundary only in the case  $n = 3$ .

We shall outline the method, merely stating results without proof. Detailed proofs will be supplied in a longer paper to appear in the near future.

It is first to be remarked that  $V_n$  is closed and bounded. Moreover it is connected; in fact, any point  $(a_2, a_3, \dots, a_n)$  of  $V_n$  can be connected to the origin  $(0, 0, \dots, 0)$  by a curve  $(a_2 t, a_3 t^2, \dots, a_n t^{n-1})$ ,  $0 \leq t \leq 1$ , each point of which lies in  $V_n$ . It is readily shown that a point  $(a_2, a_3, \dots, a_n)$  is an interior point of  $V_n$  if and only if there is a bounded  $f(z)$  belonging to  $(a_2, a_3, \dots, a_n)$ .

Write  $a_\nu = x_\nu + iy_\nu$  ( $\nu = 2, 3, \dots, n$ ). Any real function of the  $2n - 2$  coordinates  $x_2, y_2, \dots, x_n, y_n$  may be expressed as a function of  $a_2, \bar{a}_2, \dots, \bar{a}_n$ , where  $\bar{a}_\nu$  denotes the complex conjugate of  $a_\nu$ . If  $G$  is such a function we define

$$G_\nu = \frac{\partial G}{\partial a_\nu} = \frac{1}{2} \left( \frac{\partial G}{\partial x_\nu} - i \frac{\partial G}{\partial y_\nu} \right), \quad \bar{G}_\nu = \frac{\partial G}{\partial \bar{a}_\nu} = \frac{1}{2} \left( \frac{\partial G}{\partial x_\nu} + i \frac{\partial G}{\partial y_\nu} \right)$$

(provided the derivatives of first order exist). Let  $F(a_2, \bar{a}_2, \dots, a_n, \bar{a}_n)$  be defined throughout some region  $B_n$  containing  $V_n$  in its interior, and let  $F$  satisfy the following three conditions in  $B_n$ :

$$\left. \begin{array}{l} F \text{ is real;} \\ F \text{ and its derivatives of first order } F_\nu \text{ are continuous;} \\ \sum_{\nu=2}^n |F_\nu|^2 > 0. \end{array} \right\} \quad (1)$$

Suppose now that a function  $F$  of this type has its maximum value in  $V_n$  at the point  $(a_2, a_3, \dots, a_n)$ . In view of the third condition (1), we see that  $(a_2, a_3, \dots, a_n)$  must be a boundary point of  $V_n$ . If  $f(z)$  is any function belonging to  $(a_2, a_3, \dots, a_n)$ , then  $f$  maximizes  $F$  within the family of schlicht functions.

Let  $f(z)$  belong to the point  $(a_2, a_3, \dots, a_n)$ . Let  $(a, b)$  be an arbitrary analytic Jordan arc in  $|z| < 1$  but not passing through  $z = 0$ , and let  $p(z)$  be regular in a neighborhood of  $(a, b)$  and vanish at the points  $a$  and  $b$ . On pages 123–125 of "The Coefficients of Schlicht Functions, II [*Duke Mathematical Journal*, 12 (1945)] the authors showed that there is a sequence of functions

$$f_\epsilon(z) = \sum_{m=1}^{\infty} a_m(\epsilon) z^m, \quad a_1(\epsilon) = 1,$$

which are regular and schlicht in  $|z| < 1$  for  $|\epsilon| < \epsilon_0$  and such that as  $\epsilon \rightarrow 0$ ,

$$a_m(\epsilon) = a_m + \epsilon \cdot \frac{1}{2\pi i} \int_a^b p(u) \left\{ \left[ \sum_{\nu=1}^{m-1} \nu a_\nu \bar{u}^{m-\nu-2} \right] e^{-2i\tau} - \left[ \sum_{\nu=1}^{m-1} \nu a_\nu u^{-m+\nu-2} + (m-1)a_m u^{-2} - (f'(u))^2 \sum_{\nu=2}^m a_m^{(\nu)} f(u)^{-\nu-1} \right] e^{2i\tau} \right\} ds + o(\epsilon).$$

Here  $e^{i\tau}$  is the unit tangent vector of the arc  $(a, b)$  and  $ds$  is an element of arc length.

Since  $f$  maximizes  $F$ , it may be shown that  $f$  satisfies the following differential equation:

$$(zf'(z))^2 \sum_{\nu=2}^n A_\nu f(z)^{-\nu-1} = B + \sum_{\nu=1}^{n-1} (B_\nu z^{-n+\nu} + \bar{B}_\nu z^n - \nu) \quad (2)$$

where

$$A_\nu = \sum_{k=\nu}^n a_k^{(\nu)} F_k, \quad B_\nu = \sum_{k=1}^{\nu} k a_k F_{n+k-\nu}, \quad B = \sum_{k=2}^n (k-1) a_k F_k. \quad (3)$$

Here  $a_k^{(\nu)}$  are the coefficients of

$$f(z)^\nu = \sum_{k=\nu}^{\infty} a_k^{(\nu)} z^k,$$

$B$  is real, and the right side of (2) is non-negative on  $|z| = 1$  and vanishes for at least one point on  $|z| = 1$ .

It may be proved without difficulty that, corresponding to each point  $P$  of a set which is everywhere dense in the boundary of  $V_n$ , there is at least one function  $F$  satisfying conditions (1) which has an absolute maximum in  $V_n$  at  $P$ . Hence, every function belonging to a point  $P$  of this set satisfies a differential equation of type (2). But normalized schlicht functions which satisfy an equation of type (2) form a closed set, and so to every point on the boundary of  $V_n$  there belongs at least one function  $f$  which satisfies an equation of type (2).

Now let  $D$  denote any differential equation of the form (2) where  $A_2, A_3, \dots, A_n, B_1, B_2, \dots, B_{n-1}$  and  $B$  are constants such that  $B$  is real and

$$B + \sum_{\nu=1}^{n-1} (B_\nu z^{-n+\nu} + \bar{B}_\nu z^n - \nu) \geq 0$$

on  $|z| = 1$ , equality being attained for at least one point on  $|z| = 1$ .

Moreover let  $D$  be normalized by the condition that  $\sum_{\nu=2}^n |A_\nu|^2 = 1$ . A

function  $f(z)$  will be called a  $D$ -function if  $f$  is regular in  $|z| < 1$  and satisfies some  $D$  there, and if  $f$  is normalized by the conditions  $f(0) = 0, f'(0) = 1$ .

One of our main results is the following: There is a one-to-one correspondence between boundary points of  $V_n$  and  $D$ -functions. In particular, any  $D$ -function  $f(z)$  is schlicht; and  $w = f(z)$  maps  $|z| < 1$  onto the  $w$ -plane minus a portion containing  $w = \infty$  of the piecewise analytic locus which satisfies the Schiffer differential equation

$$\left(\frac{dw}{dt}\right)^2 \cdot \frac{P(w)}{w^n + 1} = -1 \quad (4)$$

where  $t$  is real and  $P(w) = A_1 w^{n-2} + A_2 w^{n-3} + \dots + A_n$ .

Let

$$F = \operatorname{Re}\{c_1 a_2 + c_2 a_3 + \dots + c_n a_n\} = \frac{1}{2} \{c_1 a_2 + \bar{c}_1 \bar{a}_2 + \dots + c_n a_n + \bar{c}_n \bar{a}_n\} \quad (5)$$

where  $c_1, c_2, \dots, c_n$  are any given complex numbers, and let the maximum value of  $F$  in  $V_n$  be  $M$ . Then  $V_n$  lies entirely on one side of the  $2n - 3$  dimensional hyperplane  $F = M$ . Since  $V_n$  is clearly non-convex for  $n > 2$ , such supporting hyperplanes can touch  $V_n$  only at points which belong to a well-defined subset of the boundary of  $V_n$ . The function  $w = f(z)$  belonging to any point of this subset of the boundary maps  $|z| < 1$  onto the  $w$ -plane minus a piece of the locus (4) on which  $P(w)$  does not vanish unless the locus lies on a straight line through  $w = 0$ . Since the boundary slits in the  $w$ -plane can have forks in the finite part of the plane only at the zeros of  $P(w)$ , we see that  $f(z)$  maps  $|z| < 1$  onto the  $w$ -plane minus one or more analytic slits meeting at  $w = \infty$ , and each of these slits is unforked.

If a function  $f(z)$  belonging to a boundary point of  $V_n$  satisfies more than one differential equation  $D$ , then the function  $f(z)$  is algebraic. This follows by dividing one  $D$ -equation by the other, the terms  $(f'(z))^2$  cancel leaving an algebraic equation in  $z$  and  $f(z)$ .

The boundary of  $V_n$  is found by prescribing conditions, involving the coefficients  $a_2, a_3, \dots, a_n$ , which insure that the solutions of equations  $D$  are regular in  $|z| < 1$ . It is, of course, necessary to make the zeros of the right side of (2) which lie in  $|z| < 1$  correspond to zeros of  $P(w)$ . We state results for the case  $n = 3$ .

Let  $V_3^{(0)}$  be the subset of  $V_3$  for which  $a_2$  is real; then  $V_3$  is the set of points  $(a_2 e^{i\theta}, a_3 e^{2i\theta})$ ,  $0 \leq \theta < \pi$ , for which  $(a_2, a_3)$  belongs to  $V_3^{(0)}$ . That is,  $V_3$  is obtained from  $V_3^{(0)}$  by rotations. The region  $V_3^{(0)}$  is symmetrical with respect to the plane  $a_2 = 0$ , so it is sufficient to describe the part of the boundary of  $V_3^{(0)}$  which lies in the half-space  $a_2 \geq 0$ . This portion of

the boundary of  $V_3^{(o)}$  is composed of the following two analytic surfaces plus their intersection:

(i) Suppose that  $0 \leq \alpha \leq \frac{\pi}{2}$ . Let  $-2(\sin \alpha - \alpha \cos \alpha) \leq \mu \leq 2(\sin \alpha - \alpha \cos \alpha)$  and define  $\lambda = 2 \cos \alpha \{\log (\cos \alpha) - 1\}$ . Then one of the two analytic surfaces is defined by

$$\left. \begin{aligned} a_2 &= \sqrt{\lambda^2 + \mu^2} \\ a_3 &= \lambda^2 + \mu^2 + 2 \cos \alpha (\lambda - i\mu) + (2 \cos^2 \alpha + 1) \frac{\lambda - i\mu}{\lambda + i\mu} \end{aligned} \right\} \quad (6)$$

Any function  $w = f(z)$  belonging to a point of this surface generally maps  $|z| < 1$  on the  $w$ -plane minus a forked slit composed of a ray amp.  $(w) = \text{constant}$  extending from  $w = \infty$  to some finite point where there is a fork composed of two prongs which form angles of  $2\pi/3$  with the ray. In special cases one or both prongs may degenerate to a point. If  $\alpha = \pi/2$ ,  $f(z)$  maps  $|z| < 1$  on the  $w$ -plane minus two rays amp.  $(w) = \text{constant}$  which make an angle of  $\pi$  at  $w = \infty$ ; in this case  $a_2, a_3$  lie on the parabola  $a_2 = \mu, a_3 = \mu^2 - 1, 0 \leq \mu \leq 2$ .

(ii) Suppose that  $0 < r < 1, -\frac{\pi}{2} \leq \varphi \leq \frac{\pi}{2}$ . Let

$$\rho^2 = 1 + 6r^2 + r^4 + 4r(1 + r^2) \cos 2\varphi,$$

$$\tan \alpha = \frac{2r \sin 2\varphi}{1 + 2r \cos 2\varphi + r^2} \quad \left( -\frac{\pi}{2} < \alpha < \frac{\pi}{2} \right)$$

$$C_1 = \frac{(1 + r)^2}{2r} \cos \varphi, \quad C_2 = \frac{(1 - r)^2}{2r} \sin \varphi$$

$$\lambda = C_1 \log \frac{\rho}{(1 + r)^2} + C_2 \alpha - 2 \cos \varphi, \quad \mu = C_2 \log \frac{\rho}{(1 - r)^2} - C_1 \alpha + 2 \sin \varphi$$

Then the portion of the boundary of  $V_3^{(o)}$  in the half-space  $a_2 \geq 0$  is completed by the surface defined by the equations

$$\left. \begin{aligned} a_2 &= \sqrt{\lambda^2 + \mu^2} \\ a_3 &= \lambda^2 + \mu^2 + (C_1 + iC_2)(\lambda - i\mu) + \left( r + \frac{1}{r} + \cos 2\varphi \right) \frac{\lambda - i\mu}{\lambda + i\mu} \end{aligned} \right\} \quad (7)$$

If  $f(z)$  belongs to a point on this boundary surface of  $V_3^{(o)}$  then  $w = f(z)$  maps  $|z| < 1$  on the  $w$ -plane minus a single curved analytic slit extending from  $w = \infty$  to some finite point. As  $r \rightarrow 0$ , the slit tends to a straight line amp.  $(w) = \text{constant}$  and the corresponding  $f$  tends to the Koebe function  $w = z/(1 + e^{-w}z)^2$ . If  $r \rightarrow 1$ , then  $C_1 \rightarrow 2 \cos \varphi, C_2 \rightarrow 0$ , and

$$\lambda \rightarrow 2 \cos \varphi \{\log (\cos \varphi) - 1\}, \quad \mu \rightarrow 2(\sin \varphi - \varphi \cos \varphi).$$

That is,  $r = 1$  corresponds to the edge of intersection of the two surfaces. For functions  $w = f(z)$  belonging to this edge the polynomial  $P(w)$  vanishes on the slit in the  $w$ -plane, but one of the two prongs of the fork is absent.



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## *STRAIN SPECIFICITY AND PRODUCTION OF ANTIBIOTIC SUBSTANCES. VII. PRODUCTION OF ACTINOMYCIN BY DIFFERENT ACTINOMYCETES\*,†*

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The ability of organisms belonging to different species or even different genera to produce the same antibiotic has been definitely established for the fungi. This is true of penicillin production by different species of *Penicillium* and *Aspergillus*; of clavacin, produced by a variety of species belonging to these two as well as other genera; of citrinin; and of other antibiotics. In the case of spore-forming bacteria, which received considerable attention during the last few years, the problem is more difficult because of the greater complexity of the substances produced. It is difficult to say as yet whether some of the products described in the literature, such as subtilin and bacitracin, are chemical individuals or mixtures. Likewise it is difficult to say whether such mixtures may contain one or more of the recognizably pure substances such as gramicidin, tyrocidine, or gramicidin S along with other active and inactive polypeptides; tyrothricin, for example, has been shown to contain gramicidin and tyrocidine, along with other less well-defined substances.

The identity of an antibiotic produced by different species of actinomycetes is complicated by the difficulty of recognizing distinct species. It is believed, however, that the data presented in this paper tend to suggest that different species, or at least what may be considered as such, may produce the same type of antibiotic. The differences in yield and especially in the impurities accompanying the particular antibiotic tend to emphasize further the differences in species, or at least in strain specificity.

In 1940, the isolation from the soil of an actinomycetes that had strong antibiotic properties was reported.<sup>1</sup> This substance was designated as actinomycin and the organism described as *Actinomyces* (*Streptomyces*) *antibioticus*.<sup>2</sup> Since then some 10,000 cultures of actinomycetes have been isolated from soils, composts, and other substrates, and tested for anti-

biotic properties, and in only two other instances was the production of this antibiotic definitely established.

The production of actinomycin can be detected by its antibiotic spectrum, by its characteristic pigmentation, and by its chemical properties, since it can be isolated from the medium and crystallized.<sup>3</sup> Its antibacterial properties comprise a very high activity against gram-positive bacteria and rather low activity against gram-negative organisms.

In a search for substances possessing activity against viruses,<sup>4</sup> a culture of actinomyces was obtained and found to produce the typical red substance described originally as actinomycin A. The new culture (S-4) resembled *S. antibioticus* in some of its morphological and cultural properties, although it was not identical with it. It produced on synthetic media, for example, a deep black zone in the aerial mycelium. The exact significance of this zone has not yet been determined.

TABLE 1  
BACTERIOSTATIC ACTION OF THREE ACTINOMYCIN PREPARATIONS

CRYSTALLINE ANTIBIOTIC	BACTERIOSTATIC ACTION BY AGAR DILUTION METHOD, DILUTION UNITS PER GRAM		
	ACTINOMYCIN FROM CULTURE S-4	ACTINOMYCIN A FROM <i>S. antibioticus</i>	ACTINOMYCIN FROM 38-G
<i>E. coli</i>	<10,000	<5,000	<30,000
<i>A. aerogenes</i>	<10,000	<10,000	<30,000
<i>S. aureus</i>	>20,000,000	>20,000,000	>3,000,000
<i>B. subtilis</i>	>20,000,000	>20,000,000	>3,000,000
<i>B. mycoides</i>	>20,000,000	>20,000,000	>3,000,000
<i>S. lutea</i>	>100,000,000	>100,000,000	.....
Bacteriostatic action by cup method, diameter of zone in mm.			
0.1 mg./ml.	31.4	31.6	
0.01 mg./ml.	27.2	27.5	

By following the original procedure<sup>3</sup> for isolating actinomycin A, a crystalline red solid was obtained which had a melting point of 252°C. and which gave no depression in the melting point when mixed with an authentic specimen of actinomycin A. The antibiotic spectra of the two substances and the quantitative concentration of pure actinomycin, as measured by the cup method against *B. subtilis*, were found to be identical, as shown in table 1.

In the course of isolation of the actinomycin from S-4, some material soluble in petroleum ether was also obtained. This fraction was previously designated actinomycin B; it was a mobile yellow oil and was produced by either strain. The bacteriostatic spectra of the two fractions (after repurification) were also similar, both giving 20,000 to 60,000 units per gram, against gram-positive bacteria. Since activity of this magnitude could result from an admixture of inert material with as little as 0.5 per cent of

actinomycin A, it is probable that the activity of this fraction is due entirely to some contamination with actinomycin A. Since even this limited activity gave exactly the same type of spectrum as actinomycin A, the second fraction may, therefore, be considered as an impurity of the latter. Because of this and in order to avoid future confusion, it is proposed to abandon the term "actinomycin B" and to change the name "actinomycin A" to "actinomycin."

The amounts of actinomycin produced by the newly isolated S-4 strain as well as by the original *S. antibioticus* 3435 strain, grown both in shaken and in stationary cultures, were then investigated. Starch tryptone medium was used, with 0.25 per cent agar for stationary cultures. The results, summarized in table 2, show that strain S-4 produces nearly 10 times as much actinomycin as the original *S. antibioticus*. The substance produced by S-4 can also be isolated more readily. The actinomycin produced by the two strains differed also in another respect: the substance formed by

TABLE 2  
PRODUCTION OF ACTINOMYCIN BY 3 DIFFERENT CULTURES OF STREPTOMYCES

STRAIN S-4		<i>S. antibioticus</i> 3435		STRAIN 36-G
SHAKEN	STATIONARY	SHAKEN	STATIONARY	STATIONARY
Activity of culture filtrate, dilution units ( <i>B. subtilis</i> )				
3,000	3,000	200	300	300
Yield of isolated crude actinomycin, milligrams per liter				
200	170	114	100	66
Total activity of crude actinomycin produced, dilution units				
1,200,000	1,000,000	140,000	120,000	250,000

S-4 was readily crystallized from acetone-ether mixtures after the removal of the B-fraction, whereas that produced by *S. antibioticus* could not be crystallized until after chromatographic separation because of the presence of tarry impurities.

In the course of this work, three methods of extraction of the actinomycin were used: (1) extraction of cultures with ether in stationary flasks; (2) continuous extraction with ether; (3) extraction with ethyl acetate. Methods 2 and 3 proved to be about equally satisfactory and were superior to method 1 on the basis of completeness of removal of the substances from the culture filtrate. The nature of the product seemed to be independent of the method of extraction.

More recently, another culture was isolated which produced actinomycin when grown on a glucose-tryptone medium, both in stationary soft agar cultures and in a submerged state. This culture, 36-G, was isolated from soil. It was markedly different from both *S. antibioticus* and S-4. It was non-chromogenic and did not form the typical sporulating aerial mycelium

characteristic of *S. antibioticus*. The straight conidiophores were arranged irregularly on the aerial mycelium. The yield of actinomycin (66 mg. per liter) given by this culture was less than that of the other 2 cultures. The activity of the culture filtrate (300 *B. subtilis* units per ml.) was similar to that of *S. antibioticus*. The antibiotic spectrum of the culture filtrate and of the crystalline product was that typical of actinomycin, as shown in the tables.

*Summary.*—Actinomycin is produced by different species of the genus *Streptomyces*. The yield and purity of the antibiotic depend upon the nature of the culture. One organism yielded about 10 times as much actinomycin as the original *S. antibioticus*. Another culture gave a lower yield than *S. antibioticus*, but a purer product was obtained. The nature and activity of the second fraction accompanying the actinomycin, namely actinomycin B, also varied for the different cultures; however, its antibiotic spectrum was similar to that of actinomycin. Because of the insignificant yields of the B fraction, and because of the suggestion that its activity is due to traces of actinomycin A present as impurities, it is proposed to abandon the name of "actinomycin B" and to change the name of "actinomycin A" to "actinomycin."

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## GROWTH REQUIREMENTS OF VIRUS-RESISTANT MUTANTS OF *ESCHERICHIA COLI* STRAIN "B"

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The isolation of a number of virus-resistant mutants from a virus-sensitive strain of *Escherichia coli* (strain B) has been described in a previous publication.<sup>1</sup> The mutant strains were obtained by using, as selective

agents, three bacterial virus strains (bacteriophages), all of which are active on the parent organism. Lysis of the sensitive cells allowed the resistant mutants to develop as secondary colonies. The mutant strains differed from the original strain by their resistance to one, any two or all three viruses, in all possible combinations. All mutants were found to possess the morphological characteristics of the parent strain. Various qualitative fermentation tests carried out on representative strains also failed to demonstrate essential differences. The mutants were tested for their ability to grow in a synthetic minimal medium containing inorganic salts, asparagine and dextrose buffered at pH 7.0. This medium will be referred to as the asparagine medium.<sup>3</sup> These tests revealed that many of the mutants were unable to develop, although the medium supported heavy growth of the parent strain. The addition of as little as 0.005 per cent Difco yeast extract to the minimal medium enabled all mutants to show good growth. In an attempt to determine what indispensable substances the mutants had apparently lost the ability to synthesize, representative strains were systematically tested in a synthetic minimal medium to which vitamins, amino acids, hydrolyzed yeast nucleic acid and hydrolyzed casein were added singly and in various combinations. The synthetic medium used in these tests contained inorganic salts, dextrose and ammonium chloride in place of asparagine, and will be referred to as the ammonium medium.<sup>3</sup> All substances tested were found to be inactive under the conditions of the tests. The present paper deals with a continuation of the investigation of the growth factor requirements of the virus-resistant mutants of strain *B*.

*Resistance Patterns of Deficient Mutants.*—The 27 mutant strains used in the present study failed to grow in either synthetic medium without the addition of nutrient broth or minute amounts of yeast extract. All deficient mutants were resistant to virus *T1*<sup>4</sup> and 22 strains were also resistant to one or more of the other viruses for which the *B* strain may serve as host. On the basis of resistance tests carried out with high titre stocks of seven viruses active on *B*,<sup>5</sup> the 27 mutants represent four different patterns of resistance as follows: 5 strains resistant to virus *T1*; 2 to *T1*, *T3* and *T4*; 3 to *T1* and *T6*; and 17 resistant to *T1*, *T3*, *T4* and *T7*. These patterns of resistance are consistent with the resistance groups discussed by Demerec and Fano.<sup>4</sup>

Seventeen of the mutants were derivatives of *B/1*, strain no. 1. These were isolated as double mutants by subjecting the *B/1* strain to the action of either virus *T2* or *T7* which, in addition to *T1*, were used as selective agents in the isolation of the mutants. Many of the derivatives of *B/1* may be the result of independent duplication of mutations. *T6* was not used in the isolation of this group of mutants. The occurrence of 3 strains resistant to *T1* and *T6* may be explained by the observation of Delbrück<sup>6</sup>



that resistance to *T*6 is facultatively coupled with resistance to *T*2. These three strains were originally isolated as *B*/2/1. It has been our experience that mutants resistant to *T*2 have a high rate of back mutation to sensitivity. In these cases it may be assumed that the organisms isolated were actually *B*/2, 6/1 and that the loss of resistance to *T*2 did not affect the resistance to *T*6 or *T*1. It has been reported<sup>4</sup> that mutants of *B* which are resistant to virus *T*1 fall into two distinct types, those which acquire resistance to *T*1 only, and those which simultaneously acquire resistance to *T*5. All the strains unable to grow in synthetic media were found to be sensitive to *T*5.

*Tryptophane as Growth Factor and the Influence of Nitrogen Source.*—All substances tested in the previously reported studies<sup>1</sup> gave completely negative results with the exception of *dl*-tryptophane which, in a concentration of 1 microgram per milliliter, gave inconclusive growth with the mutant strains used in the tests. On testing the 27 deficient strains for their ability to grow in the ammonium medium containing 30 micrograms *l*(—)-tryptophane per milliliter it was found that, with one exception, all strains showed some development after 14 hours' incubation at 37°C. and good growth at the end of 38 hours. Other amino acids<sup>7</sup> added to the ammonium medium in concentrations of 100 micrograms per milliliter failed to allow development of any of the mutant strains tested.

In an attempt to determine the concentration of *l*(—)-tryptophane necessary for development of *B*/1, strain no. 1, a graded series of tryptophane concentrations ranging from 200 to 0.01 microgram per milliliter was set up in 1-ml. amounts in the ammonium medium and in the asparagine medium. After 20 hours' incubation at 37°C. visible growth was observed in the ammonium medium in all tubes containing 20 micrograms of tryptophane while the series in the asparagine medium showed growth at 0.2 microgram per milliliter. Incubation over a period of 140 hours permitted visible growth in the ammonium series to 1.56 micrograms tryptophane per milliliter. Development in this medium, however, was greatly reduced in all tubes containing less than 12.5 micrograms of tryptophane per milliliter and in the asparagine medium at concentrations of less than 1.13 micrograms of tryptophane per milliliter.

Comparative studies on the growth of the parent strain in the two synthetic media had shown  $\text{NH}_4\text{Cl}$  and asparagine to be entirely comparable as a nitrogen source. The observation that the asparagine medium permitted growth of the deficient strain at a much lower concentration of tryptophane than did the ammonium medium indicated that the deficient strains, in addition to requiring tryptophane as a growth factor, may have nitrogen requirements which differ from those of the parent strain. Therefore, a more extensive investigation of the nitrogen requirements of the deficient and the parent strains was undertaken.



*Amino Acids as Nitrogen Source in Combination with  $\text{NH}_4\text{Cl}$ .*—Preliminary studies were carried out with *B/1*, strain no. 1, in which 21 amino acids were individually tested for their ability to serve as an additional nitrogen source when added to the ammonium medium supplemented with tryptophane. Each amino acid was tested at a concentration of 100 micrograms per milliliter in two series of media, one of which contained 20 micrograms *l*(-)-tryptophane per milliliter and the other 100 micrograms per milliliter. The tests were carried out in 10-ml. volumes in large test tubes with aeration. Growth was measured after 24 hours' incubation at 37°C. by centrifuging each culture in its entirety in Hopkins' vaccine tubes. Nearly all amino acids tested gave some increase in the cell yield of the organism over that obtainable in the controls containing tryptophane and  $\text{NH}_4\text{Cl}$  as sole nitrogen source. An exception was *dl*-norleucine which inhibited growth at the concentration tested. Ten amino acids, however, permitted appreciably increased growth of *B/1*. These were *l*(-)-histidine, *l*(+)-arginine, *l*(-)-leucine, *dl*-phenylalanine, *dl*-valine, glycine, *dl*-serine, *l*(+)-glutamic acid, *l*(-)-proline and *l*-asparagine.

*Selected Amino Acids as Sole Nitrogen Source for the Parent Strain.*—These ten amino acids as well as *l*(-)-tryptophane were tested as sole nitrogen source for the parent strain. They were added in 1 mg. per milliliter amounts to 10-ml. volumes of the minimal medium with the  $\text{NH}_4\text{Cl}$  omitted. Cell volume measurements of *B*, determined as above, indicated that the eleven amino acids tested could be arbitrarily divided into three groups on the basis of their ability to serve as nitrogen sources for strain *B*. Group I, consisting of asparagine, arginine, serine and glycine gave good growth. Proline and glutamic acid in group II gave fair growth. Very little or no growth was obtained with histidine, tryptophane, leucine, valine and phenylalanine which comprised group III. The inability of the parent strain to develop on group III amino acids cannot be considered as due to the necessity of accessory growth factors since it is fully able to grow in inorganic nitrogen and glucose. Parallel tests in which the amino acids were added to the minimal medium containing  $\text{NH}_4\text{Cl}$  showed that all eleven amino acids permitted good growth in the presence of the ammonium salt. Therefore, the failure of the group III amino acids to permit growth when present as sole nitrogen source may be explained on the basis that their nitrogen is unavailable rather than that these amino acids are toxic to the organism. The data obtained in one representative experiment are presented in table 1. It will be noted that although tryptophane is essential for the growth of *B/1*, it is not a suitable source of nitrogen for *B* in the absence of other nitrogen sources.

*Selected Amino Acids as Sole Nitrogen Source for *B/1*.*—Essentially the same grouping was obtained when the ten amino acids were tested individually for their ability to serve as sole nitrogen sources for *B/1* in the

minimal medium containing no  $\text{NH}_4\text{Cl}$ . The tests with this organism were carried out in the same manner as those for the parent strain except that 100 micrograms tryptophane per milliliter were added as a growth factor. Although cell yields of *B/1* are low with  $\text{NH}_4\text{Cl}$  as sole source of nitrogen, it is apparent that  $\text{NH}_4$ -nitrogen may be used in the presence of certain amino acids. As is seen in table 1,  $\text{NH}_4\text{Cl}$  appears to be essential for the utilization of histidine, leucine, valine and phenylalanine by *B/1*. It is not clear whether  $\text{NH}_4$ -nitrogen is used in the presence of the other amino acids since growth is heavy in its absence. It was observed, however, that in all cases visible growth was obtained earlier in tubes containing  $\text{NH}_4\text{Cl}$  than in parallel tubes in which this salt was omitted.

TABLE 1

GROWTH OF *B*, *B/1*, STRAIN NO. 1 AND *B/1/7*, 3, 4, STRAIN NO. 1A, ON SELECTED AMINO ACIDS IN A MINIMAL MEDIUM WITH AND WITHOUT  $\text{NH}_4\text{Cl}$

SUBSTANCE ADDED, 1 MG. PER ML.	<i>B</i>		<i>B/1</i> , NO. 1		<i>B/1/7</i> , 3, 4, NO. 1A	
	- $\text{NH}_4\text{Cl}$	+ $\text{NH}_4\text{Cl}$	- $\text{NH}_4\text{Cl}$	+ $\text{NH}_4\text{Cl}$	- $\text{NH}_4\text{Cl}$	+ $\text{NH}_4\text{Cl}$
<i>l</i> (+)-Arginine	41.5	46.0	26.0	33.0	20.0	27.0
<i>l</i> -Asparagine	29.0	28.0	17.0	12.0	15.0	30.0
<i>dl</i> -Serine	36.0	36.0	26.5	30.0	26.5	26.5
Glycine	26.5	30.0	12.0	17.0	15.0	20.5
<i>l</i> (-)-Proline	5.0	26.0	8.0	12.0	9.0	20.0
<i>l</i> (+)-Glutamic acid	3.5	29.5	11.0	16.5	7.5	18.0
<i>l</i> (-)-Histidine	0.0	19.0	0.5	30.5	3.5	23.0
<i>l</i> (-)-Tryptophane	0.5	26.5				
<i>l</i> (-)-Leucine	0.5	22.5	1.0	11.0	7.0	15.0
<i>dl</i> -Valine	1.0	32.0	1.0	19.5	6.0	24.0
<i>dl</i> -Phenylalanine	0.5	22.5	0.5	15.0	6.0	24.0
Control	0.0	24.0	0.5	3.5	3.5	9.0

Growth in mm.<sup>3</sup> cells per 10 ml. in 24 hours.

Media used in testing *B/1* contained 100 micrograms *l*-tryptophane per milliliter.

Media used in testing *B/1/7*, 3, 4 contained 100 micrograms *l*-tryptophane and 100 micrograms *l*-proline per milliliter.

*Tryptophane Requirements with Optimal Nitrogen Source.*—It is apparent from these results that the amount of growth of the tryptophane requiring strains is limited not only by the amount of tryptophane present in the medium, but also by the nature of the substances serving as source of nitrogen. Therefore, in testing the efficiency of *l*(-)-tryptophane as a growth factor for the deficient strains the tests were carried out in the ammonium medium to which 1 mg. of hydrochloric acid-hydrolyzed yeast extract per milliliter was added. This preparation was entirely free of tryptophane but together with  $\text{NH}_4\text{Cl}$  served as a completely adequate source of nitrogen.

Figure 1 presents the growth response of *B/1*, strain no. 1, to varying concentrations of *l*(-)-tryptophane in this medium. The determinations

were carried out in 1-ml. volumes in small test tubes without aeration and cell counts were made by the usual plating method at the end of 24 hours' incubation at 37°C. From the data presented it is seen that, provided a suitable nitrogen source is available in sufficient quantity, the growth of this mutant is proportional to the amount of tryptophane added and that with 0.25 microgram of *l*(-)-tryptophane  $10^8$  cells developed in 24 hours.

*Irreplaceability of Tryptophane.*—In the case of certain microorganisms which require tryptophane for growth it has been shown that this amino acid can be replaced by substances postulated as intermediate in the synthesis of tryptophane. Indole is utilized by some strains of *B. typhosum*,

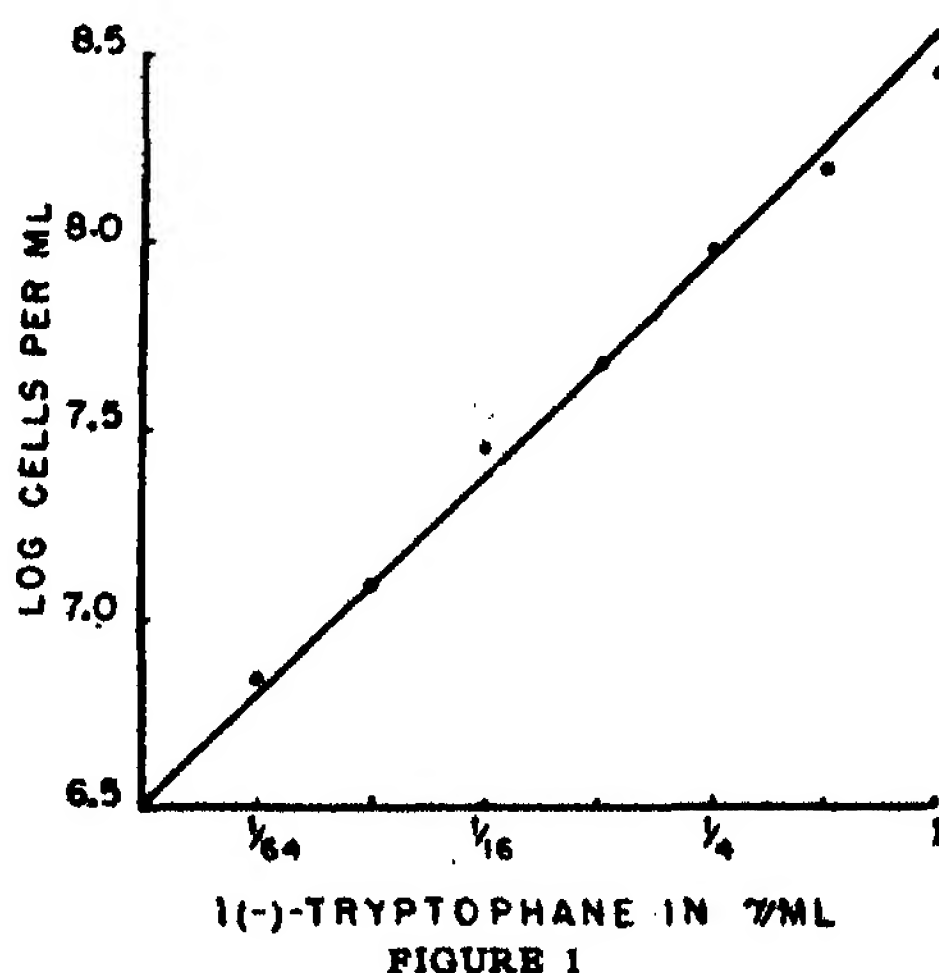


FIGURE 1  
Growth of *B*/1, no. 1 in varying concentrations of *l*(-)-tryptophane.

*C. diphtheriae* and staphylococcus,<sup>8</sup> and either indole or anthranilic acid can replace tryptophane for certain species of lactic acid bacteria.<sup>9</sup> Two tryptophaneless mutant strains of the ascomycete *Neurospora crassa* have been described,<sup>10</sup> one of which is able to use indole and the other either indole or anthranilic acid in place of tryptophane. Tatum and Bonner<sup>11</sup> have presented evidence that in this mold the biosynthesis of tryptophane takes place by a direct reaction between indole and *l*(-)-serine.

Eight tryptophaneless mutants of *B* were tested for their ability to grow in the ammonium medium, supplemented by 1 mg. per milliliter hydrochloric acid-hydrolyzed yeast extract, to which was added *l*(-)-tryptophane, indole,<sup>12</sup> or anthranilic acid<sup>12</sup> in 50 micrograms per milliliter amounts. Additional tests were carried out using *B*/1, strain no. 1, in the ammonium medium to which was added alpha methyl indole,<sup>13</sup> xanthurenic acid,<sup>13</sup>

tryptamine hydrochloride,<sup>12</sup> indole acetic acid,<sup>12</sup> indole propionic acid,<sup>12</sup> indole pyruvic acid,<sup>12</sup> indole butyric acid<sup>12</sup> or indole + *dl*-serine. In no case was growth of any of the tryptophane-deficient mutants of *B* obtained in the absence of tryptophane.

With one exception tryptophane has been found to satisfy the growth factor requirements of all mutants unable to grow in either minimal medium. In addition, tryptophane is required in much higher concentrations when  $\text{NH}_4\text{Cl}$  is supplied as the nitrogen source than the concentration necessary to support good development in the presence of certain amino acids.

All strains are capable of development in the ammonium medium supplemented with small amounts of tryptophane and hydrochloric acid-hydrolyzed yeast extract. This combination of nutrilites was found to satisfy the growth requirements of the one strain, *B*/1/7, 3, 4, no. 1A, which was unable to grow in the ammonium or asparagine medium supplemented with tryptophane alone. Since *B*/1/7, 3, 4, no. 1A, is incapable of development in synthetic media supplemented with the hydrolyzed yeast extract alone, it is apparent that this strain requires tryptophane as well as some factor or factors present in yeast hydrolyzate.

*Proline and Tryptophane Required by B*/1/7, 3, 4, No. 1A.—In order to determine whether this strain requires other amino acids as growth factors in addition to tryptophane, 1-milliliter amounts of the ammonium medium containing 100 micrograms of *l*(-)-tryptophane per milliliter and 100 micrograms of individual amino acids per milliliter were set up in small test tubes and incubated at 37°C. without aeration. The only tubes showing development at the end of 24 hours were those containing tryptophane plus *l*(-)-proline and tryptophane plus *l*(-)-hydroxyproline. Incubation for 144 hours resulted in a slight increase of those two tubes but no visible growth with any of the other 19 amino acids tested. Much higher cell concentrations were obtained with proline than with hydroxyproline. The optimum concentrations of tryptophane and proline required for the growth of this strain have not been determined.

*Selected Amino Acids as Sole Nitrogen Source for B*/1/7, 3, 4, No. 1A.—The tryptophane-proline-deficient mutant was also tested for its ability to utilize the group of ten amino acids as sole nitrogen sources. Tests were carried out as for *B* and *B*/1, but with tryptophane and proline, 100 micrograms of each per milliliter, added as growth factors to the minimal medium. From the data presented in table 1 it is seen that the amino acids fall into essentially the same grouping as with the other two strains. Growth in the control is, however, greater than that in the controls of *B*/1. This may be due to the presence of proline in an amount above the growth factor requirements for this amino acid, the excess being utilized as nitrogen source. This is reflected in the growth of this strain with most of the group III amino acids in the media without  $\text{NH}_4\text{Cl}$ . The addition

of  $\text{NH}_4\text{Cl}$  to the media permitted good development of *B/1/7*, 3, 4, strain no. 1A, with all amino acids tested.

*Discussion.*—Certain mutations from virus sensitivity to virus resistance in the *B* strain of *E. coli* may result in a loss of the ability of the mutant to synthesize factors essential for growth. This is indicated by the observation that many of the mutant strains are unable to develop in the absence of tryptophane. In the present study all tryptophaneless mutants have been found to be resistant to *T1* but sensitive to *T5*. Mutants resistant to *T1* and *T5* are capable of growth with inorganic nitrogen and glucose and therefore may be presumed to have retained the synthetic capacity of the parent strain. It would therefore appear that resistance to *T1* may be the result of different mutations—one type blocking the synthesis of an essential metabolic product in addition to preventing the synthesis of specific constituents essential to the reaction of the cell and a specific virus. Further studies, however, will be required to determine whether or not the loss of the capacity of synthesizing tryptophane is directly correlated with a specific resistance pattern. The deficient strain requiring proline in addition to tryptophane was one of 17 *B/1/7*, 3, 4 strains. Tryptophane alone satisfied the need for accessory growth factors for the other 16 strains, indicating that there are different mutations involving resistance to *T7* and that the need for proline may be associated with one specific type of these mutations.

That other factors in the synthetic capacity of the tryptophaneless mutants may also become limiting as a result of the mutation is indicated by the observation that even though the growth factor requirements be satisfied, the deficient mutants, in contrast to the parent strain, are incapable of growing to high cell concentrations when  $\text{NH}_4\text{Cl}$  is supplied as sole source of nitrogen. Supplementation with amino nitrogen results in greatly increased growth. Therefore it would appear that the amination capacity of these strains has become altered. In this respect it is interesting that the presence of  $\text{NH}_4$ -nitrogen appears to be essential for the utilization of certain of the amino acids.

*Summary.*—Certain virus-resistant mutants derived from a virus-sensitive strain of *E. coli* were found to require accessory growth factors although the parent strain is capable of full development in a medium containing inorganic nitrogen and dextrose. The 27 strains used in this study have apparently lost the ability to synthesize tryptophane. One strain was found to require proline in addition to tryptophane. All of the tryptophaneless strains are resistant to virus *T1* and sensitive to *T5*.

That other factors in the synthetic capacity of the mutants differ from those of the parent strain is evidenced by the observation that organic as well as ammonia nitrogen is essential for full development of the mutant strains. In a medium containing adequate nitrogen sources the addition



of 0.25 microgram of *l*(-)-tryptophane gives a development of  $10^8$  cells in 24 hours.

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<sup>1</sup> Anderson, E. H., these PROCEEDINGS, 30, 397-403 (1944).

<sup>2</sup> The asparagine medium consisted of: *l*-asparagine 0.2 per cent, dextrose 0.4 per cent,  $\text{Na}_2\text{HPO}_4$  (anhydrous) 0.6 per cent,  $\text{KH}_2\text{PO}_4$  0.3 per cent,  $\text{MgSO}_4$  0.005 per cent,  $\text{NaCl}$  0.005 per cent, glass distilled water.

<sup>3</sup> The ammonium medium consisted of:  $\text{NH}_4\text{Cl}$  0.1 per cent, dextrose 0.4 per cent,  $\text{NaHPO}_4$  (anhydrous) 0.6 per cent,  $\text{KH}_2\text{PO}_4$  0.3 per cent,  $\text{MgSO}_4$  0.02 per cent,  $\text{NaCl}$  0.05 per cent, glass distilled water.

<sup>4</sup> Demerec, M., and Fano, U., *Genetics*, 30, 119-136 (1945).

<sup>5</sup> Delbrück, M., *Biological Reviews*, 21, 30-40 (1946).

<sup>6</sup> Delbrück, M., unpublished data.

<sup>7</sup> The following amino acids were tested: *dl*-alanine, *l*(+)-arginine, *l*-asparagine, *l*(+)-aspartic acid, *l*(-)-cystine, *l*(+)-glutamic acid, glycine, *l*(-)-histidine, *l*(-)-hydroxyproline, *dl*-isoleucine, *l*(-)-leucine, *dl*-leucine, *l*(+)-lysine, *dl*-methionine, *dl*-norleucine, *dl*-phenylalanine, *l*(-)-proline, *dl*-serine, *dl*-threonine, *l*(-)-tryptophane, *l*(-)-tyrosine, *dl*-valine.

<sup>8</sup> Fildes, P., *Brit. Jour. Exptl. Path.*, 21, 315-319 (1940).

<sup>9</sup> Snell, E. E., *Arch. Biochem.*, 2, 389-394 (1943).

<sup>10</sup> Tatum, E. L., Bonner, D., and Beadle, G. W., *Ibid.*, 3, 477-478 (1944).

<sup>11</sup> Tatum, E. L., and Bonner, D., these PROCEEDINGS, 30, 30-37 (1944).

<sup>12</sup> Kindly provided by Dr. E. L. Tatum, Osborn Botanical Lab., Yale Univ.

## EXPERIMENTS ON SEXUAL ISOLATION IN DROSOPHILA. VII. THE NATURE OF THE ISOLATING MECHANISMS BE- TWEEN DROSOPHILA PSEUDOOBSCURA AND DROSOPHILA PERSIMILIS

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An ever increasing number of cases is being described in the current literature of pairs of exceedingly similar species that coexist at the same locality. The morphological similarity sometimes reaches the point of virtual identity, in other cases very minor differences exist in regard to the characters of eggs, larvae or adults. Adherents of a strictly morphological species concept interpret such findings as intraspecific differentiation and apply the term "physiological races" to the members of such pairs of extremely similar species. However, it has been found in all well-studied



cases that partial or complete reproductive isolation exists between the members of these pairs of populations and that no hybrids are found in nature in the regions of distributional overlap, even in the cases where hybrids can be obtained experimentally. The adherent of a biological species concept is forced to regard as species sympatric populations that are reproductively completely isolated. The two species *Drosophila pseudoobscura* and *D. persimilis* are such a pair of species. Although the morphological differences between these species<sup>1</sup> are very slight, still not a single hybrid has yet been found in the wide area of overlap in western America. In the laboratory the two species can be crossed fairly easily, and the hybrid females are fertile and can be backcrossed. Hybrid  $F_1$  males, however, are completely sterile.<sup>2</sup>

*Sex Behavior in Intraspecific Crosses.*—A study of the normal sex behavior of the two species would seem a necessary prerequisite of a study of their mutual incompatibilities and isolating mechanisms. Unfortunately, however, the sex physiology of the two species is by no means exhaustively known.

Some data are presented in the following sections on the sexual physiology and the isolating mechanisms of an orange-eyed strain of *D. pseudoobscura* Frolova descended from flies collected at Piñon Flats, San Jacinto Mountains, California, and a wild strain of *D. persimilis* Dobzhansky and Epling from Stony Creek, north of the Sequoia National Park, California. Only these two strains of the two species were studied. Courtship in *D. melanogaster* and many other species has been described by Sturtevant<sup>3, 4, 5</sup> and in *D. virilis* by Stalker.<sup>6</sup> For *D. pseudoobscura* and relatives data were presented by several authors.<sup>2, 7, 8</sup>

*Age at Sexual Maturity.*—Flies of the species *D. pseudoobscura* become sexually mature earlier than flies of the closely related species *persimilis* and *miranda*. Dobzhansky and Koller<sup>7</sup> found that in a strain of *D. pseudoobscura* kept at 24.5°C. about 36% of the females were fertilized after 1½ days, 62% after 2 days, 82% after 3 days. In a strain of *D. miranda* only 4% of the females were fertilized after 2 days, 56% after 3 days, 66% after 4 days. *D. persimilis* seems to be still slower. Even 3-day-old flies are rarely fertilized and flies at least 5 days old (preferably 6 or 7) have to be used, to be sure that they are sexually mature. This poses the awkward problem that flies of different chronological age must be used to be sure that they are approximately of the same physiological age. Therefore the females of *D. pseudoobscura* used for most of the experiments were 5 or 6 days old and those of *persimilis* 7 days old.

*Sexual Activity.*—Both males and females of the orange-eyed strain of *D. pseudoobscura* were sexually much more active than flies of the *D. persimilis* strain. It did not prove feasible to measure this difference quantitatively, but if the following arbitrary point values are given to the sex drives:

♂ *pseudoobscura* 120, ♀ *pseudoobscura* 80, ♂ *persimilis* 50, ♀ *persimilis* 10, and a value of  $-110$  to the isolating mechanisms in the interspecific matings, we obtain the following combined values:

$$\textit{persimilis} \text{ ♂ with } \textit{persimilis} \text{ ♀ : } 50 + 10 = 60$$

$$\textit{persimilis} \text{ ♂ with } \textit{pseudoobscura} \text{ ♀ : } 50 + 80 - 110 = 20$$

$$\textit{pseudoobscura} \text{ ♂ with } \textit{pseudoobscura} \text{ ♀ : } 120 + 80 = 200$$

$$\textit{pseudoobscura} \text{ ♂ with } \textit{persimilis} \text{ ♀ : } 120 + 10 - 110 = 20$$

The ratios 60:20 ( $=3:1$ ) and 200:20 ( $=10:1$ ) are fairly close to the observed ones in double choice experiments.<sup>8</sup> They are not entirely exact, however, since the actual values of the isolating mechanisms are fairly different in reciprocal crosses (above listed always as  $-110$ ). The incompatibility between *pseudoobscura* ♂ and *persimilis* ♀ is much greater than that between *persimilis* ♂ and *pseudoobscura* ♀.

Sexual activity in *Drosophila*, unfortunately, happens to be a somewhat unpredictable factor, particularly in well-aged flies. It was high on certain days, low on others even though all experimental conditions including temperature were seemingly identical. The time of day seemed to play some rôle: sexual activity is apparently higher in the morning and evening than during the middle of the day. Quantitative experiments, to be strictly comparable, should be conducted not only at the same temperatures but also at the same hour of the day.

*Mating Behavior of Males.*—It has been shown by Sturtevant<sup>3, 4, 5</sup> that the following elements are the most frequent components of the mating behavior of *Drosophila* males. In various combinations they are found in most species of the large genus *Drosophila*.

"*Vibrating.*"—The male faces the female (usually from the side), extends one wing at about right angles to his body, and vibrates it for a few seconds. The wing is then returned to the normal position. The vibrated wing is usually the one nearer the head of the female. Both wings are rarely vibrated simultaneously and only in a few species.

"*Waving.*"—The wing is also extended laterally, but is not vibrated.

"*Scissors Movements.*"—Both wings are rapidly opened and closed like a pair of scissors.

"*Licking.*"—The male licks with his proboscis the ovipositor of the female.

"*Circling.*"—A rapid sideways movement of the male from a frontal or lateral position to one behind the female (usually followed immediately by copulation).

Of these five elements only *vibrating* and *circling* were found in *D. pseudoobscura* and *persimilis*. It was not possible to discover any difference between the two species, either in the qualitative or quantitative aspects of the courtship.

Copulation in the two species proceeds as described by Sturtevant<sup>3</sup> for *D. melanogaster*. The male, standing behind the female, bends up his abdomen underneath, until its tip faces forward. The phallus is then thrust into the female ovipositor, and after its intromission the male parts the wings of the female and mounts. In some other species the female opens her wings spontaneously and in still others the male mounts the back of the female before intromission of the phallus.

Termination of a normal copula is apparently always initiated by the male by extracting his phallus. Usually he succeeds in doing this in 20 or 30 seconds, but it may require 3 or 4 minutes in exceptional cases. Males of the observed strain of *D. persimilis* were usually rather inactive after completed copulation and spent much of their time in preening. No second copulation was recorded during the observation periods. Males of the observed strain of *D. pseudoobscura* sometimes engaged in a second copulation within 30 or 40 seconds after completion of the first, and in a third copulation after completing the second. If no receptive females are available, males may become completely quiescent within about 20 minutes after a period of great excitement and much displaying.

*Female Behavior.*—Receptive females stand still, turn the tip of the abdomen toward the male, lift it and partly extrude the ovipositor ("invitation display"). With ready males this will result in almost instantaneous copulation, other males—particularly young males and males of other species—may pay no attention to the female's overtures. Many males copulate without a preceding invitation display by the female. Copulating females ward off other males by stretching the middle pair of legs sideways. Non-receptivity is indicated by the following actions of females: walking away rapidly, wing-flicking, depressing the tip of the abdomen toward the ground, or a combination of these methods. Females of *D. pseudoobscura* and *D. persimilis*, which had just completed copulation, were non-receptive at least for one hour. They were receptive when tested again 24 hours later. Sturtevant<sup>4</sup> found that in *D. repleta* and *affinis* the same pair may copulate twice within 10 minutes. Repeated copulations were also found in other species.

*Length of Copulation.*—Sturtevant<sup>4,5</sup> reports that the length of copulation of various species of *Drosophila* may vary between 1 minute (*lutzii*, *hydei*) and 55 minutes (*immigrans*).

First copulations of *D. pseudoobscura* males lasted 4'30", 4'35", 4'55", 5'55", 6'10", 6'15", 6'15", 7'0", 7'10", 8'0", 9'15", 10'5", 11'15" in homogamic matings (median 6'15"). No copulations with *D. persimilis* ♀ were timed. Second and third successive copulations usually last shorter than the first. The duration of successive copulations was: 4'30", 3'26", 2'20"; —4'55", 3'10", 4'15", —7'10", 4'40"; —8'0", 5'20". Temperatures were about 21–24°C., but unfortunately were not accurately recorded.

First copulations of *D. persimilis* males lasted 4'40", 5'0", 5'35", 5'40", 6'10", 6'10", 6'10", 6'25", 6'35", 6'40", 7'0", 7'20", 7'20", 8'20", 8'30", 9'25" in homogamic matings (median 6'30"). One copulation with a *pseudoobscura* female lasted 7'35". There is thus no striking difference between the two species. Stalker<sup>6</sup> likewise found no significant difference in length of copulation between the closely related species *D. virilis* and *D. americana*.

*Species Recognition and Psychological Isolating Mechanisms.*—Most modern authors assume that "psychological barriers" ("species recognition") prevent or reduce the frequency of matings between members of closely related species of insects. These terms signify a crude concept of the interplay between male and female, which has no reality. The term "recognition" implies consciousness and the ability of making judgments, for which no evidence exists in *Drosophila*. Rather it must be assumed that the male stimulates the female by specific pre-copulatory displays and that the female reacts by specific responses indicating a state of receptiveness. This interpretation assumes that successful copulation is the result of a chain of interactions between specific stimuli produced by the male and adequate responses of the female which in turn stimulate the male.

If the reproductive isolation between *D. pseudoobscura* and *D. persimilis* is partly or entirely due to psychological isolating mechanisms, an analysis of the pre-copulatory display of males and females should reveal differences. The above-described observations indicate that there are no visible differences in the courtship behavior of the two species. This is, in a way, not surprising since the major elements of the courtship, vibrating, circling, scissors movement, and licking in various combinations are widespread in the genus *Drosophila*.

The possibility remains that auditory, olfactory or other factors provide the stimulation necessary to limit copulation to encounters between conspecific individuals. To test this possibility a series of multiple choice experiments were undertaken.<sup>8</sup>

*Multiple Choice Experiments.*—Males of one species were given the opportunity to mate under varying conditions with females of two species. When an equal number of females of *D. pseudoobscura* and *D. persimilis* was placed in a vial with food together with males of *D. pseudoobscura*, it was found<sup>8</sup> that about 11 times as many *pseudoobscura* females were inseminated as *persimilis* females. Males of *persimilis* fertilized about 3 times as many of their own as *pseudoobscura* females. These control experiments, as well as those of earlier authors,<sup>2, 7</sup> permit three conclusions. First, that sexual isolation between the two species is not nearly as complete under experimental conditions (only one kind of male present) as in nature. Second, that mating between the flies is not random, but indicative of highly developed discrimination. Third, that conspecific matings are much more



frequent than heterogamic matings. The experiments, however, do not elucidate the reason for the higher frequency of conspecific pairings.

Questions that need to be answered are the following: Is a fly stimulated by an individual of the opposite sex regardless of the species to which it belongs? If there is a difference between species in stimulation, how large is it and to what extent is it responsible for the reproductive isolation of the species concerned? Are male and female equally involved in the difference which seems to exist between conspecific and non-specific pre-copulatory stimuli? Which sense organs are important as receptory mechanisms for these stimuli?

*Methods.*—Different techniques were employed in the attempt to elucidate these questions. In mass experiments 10 females of each of two species were placed in a vial of food with several males of one of these species. The females were dissected after an interval sufficient to permit fertilization of about 50 per cent of them, and the percentage of fertilized females in the lots of the two species determined. This indirect method was supplemented by direct observation. A special observation chamber was constructed which consisted of a wax ring between two parallel glass plates. The size of the ring was adjusted not to exceed the field of vision of a low-power binocular microscope. The flies were introduced into the wax ring through a funnel-like opening which could be closed by a stopper. In this chamber flies remained in good physical condition for hours, but most observation periods were terminated after 30 minutes and the flies replaced by new ones. This observation chamber permitted the observation at a 7-fold magnification of every detail of the movements of 4–8 flies, all of them at all times completely in focus.

A different observation technique was employed where numerical counts were more important than a study of the details of behavior. Batteries of ten glass vials without food were used, each one of them containing the same combination of flies. The ten vials were observed simultaneously and the number and sequence of events recorded. All transfers of aged flies were made without etherization. All tested flies were virgin at the beginning of the observation periods.

*The Role of the Sense Organs.*—*Vision:* The two species *D. pseudoobscura* and *D. persimilis* are indistinguishable to the human eye. There is no significant difference in the insemination ratio of mixed cultures kept in the light and such that were kept in the dark.<sup>8</sup> This indicates that vision is not essential for species discrimination. However, absolute proof for the rôle of light can be obtained only if the dark-light experiment could be repeated after the complete elimination of all other sense organs, because it is conceivable that other senses might take over the function of vision after its elimination.

*Hearing:* When a male of *D. pseudoobscura* or *D. persimilis* courts a

female he spreads a wing and vibrates it. Since Reed, *et al.*,<sup>9</sup> have shown that the means of the wing areas are different in the two species, the possibility exists that the pitch of wing vibration is also different and may serve as a "species recognition signal." However, it was shown<sup>8</sup> in experiments involving wingless males, that actually a smaller percentage of alien females was inseminated and that the total number of inseminated females had dropped. It seems on the basis of these and other observations that it is the rôle of the vibrating wings to stimulate the females and to get them into a receptive state. Furthermore, the overlap in the normal wing pitch variability of the two species is much too large for a character to be useful in species discrimination.

*Smell:* It is well known that specific scents play an important rôle in the courtship of many insects. It was therefore tried to test what effect on species discrimination the elimination of the olfactory sense would have. The olfactory organ of *Drosophila* is located in the terminal segment of the antennae,<sup>10, 11</sup> and can be removed rather easily. Four males of *D. pseudoobscura*, each placed with ten females of *D. pseudoobscura* and *D. persimilis* one day after the complete amputation of all segments of both antennae, performed as shown in table 1.

TABLE 1  
RECORDS OF *D. pseudoobscura* AND *D. persimilis* FEMALES INSEMINATED BY  
*D. pseudoobscura* MALES WITHOUT ANTENNAE

HOMOGAMIC FEMALES		HETEROGAMIC FEMALES		ISOLATION INDEX
N	%	N	%	
38	52.7	39	10.2	0.68

N = number of females; % = percentage of inseminated females.

Although the isolation index is significantly lower than in control experiments<sup>8</sup> (where it is 0.80 or higher), still five times as many conspecific as alien females were inseminated by males without antennae. If these findings could be confirmed with more extensive material, they would indicate the following facts: lack of the olfactory sense in males increases the number of heterogamic crosses, which implies that to a certain extent the olfactory sense of males is involved in species discrimination. However, species discrimination is still high even without the olfactory apparatus.

*Absence of Species Discrimination in Courting Males.*—The reported experiments indicate that none of the investigated sense organs and display mechanisms had a controlling influence on species discrimination. It appeared therefore advisable to record quantitative data on species discrimination by direct observation of courting males, both in the observation chamber and in the vial batteries previously described. The observations gave no indication of species discrimination by males, as documented by the following excerpts from my protocols. (The term "incomplete copula-



tions" is applied to copulations which are typical in every respect and include intromission of the male phallus and mounting, but are terminated after 1-2 seconds.)

"July 19 (10:15 A.M.). . 2 ♂ *pseudoobscura* (8 days old) placed with 4 ♀ *persimilis* (6 days old). Males very active and aggressive. No less than 50 incomplete copulations observed during a 30-minute period. No sperm found in genital tract of females. Females seem to coöperate fully during the incomplete copulations. They go through the same motions as when being courted by their own males, such as stopping, lifting the abdomen and turning it slightly toward the courting male."

Males display to alien females and attempt to copulate with them even when females of their own species are available. This is particularly true when males of *D. persimilis* are placed with the very active *pseudoobscura* females together with their own rather quiet and sluggish females:

"July 20 (10:26 A.M.). 3 ♂ *persimilis* (8 days old), 3 ♀ *persimilis* (8 days old), 2 ♀ *pseudoobscura* (7 days old). 30-minute period. Male *persimilis* rather active, display both to *persimilis* and *pseudoobscura* females. After 15 minutes first and only copulation (homogamic). Males display during the last fifteen minutes almost entirely to *pseudoobscura* females."

"July 21 (8:06 A.M.). One hour. 3 ♂ *persimilis* (8 days old), 3 ♀ *persimilis* (8 days old), 3 ♀ *pseudoobscura* (8 days old). There are numerous incomplete copulations of *persimilis* males with *pseudoobscura* females. One such heterogamic copulation is successful. During the whole hour there is not a single persistent attempt of a *persimilis* ♂ to copulate with a *persimilis* ♀."

"August 2 (7:42 P.M.). One hour. Ten vials each with 1 *persimilis* ♂ (7 days old), 1 *persimilis* ♀ (7 days old), 1 *pseudoobscura* ♀ (6 days old). During first 10 minutes males display almost exclusively to *pseudoobscura* females. In three vials there are very frequent incomplete copulations with *pseudoobscura* ♀. The males in two vials are entirely inactive, in six of the other vials they clearly concentrate their attention on *pseudoobscura* females. However, not a single successful heterogamic copulation occurred. The only attempted homogamic copulation was at once successful. The *persimilis* ♀ of one vial walked repeatedly past the *persimilis* ♂, who persistently displayed to *pseudoobscura* ♀ and payed no attention to his own female."

All female flies were dissected 13 hours after end of observation. Five *persimilis* ♀ and one *pseudoobscura* ♀ were found to be inseminated.

These records show clearly that males display without apparent discrimination to females of both species, and that in fact the majority of the display of *persimilis* ♂ are directed toward *pseudoobscura* ♀, which are more active than their own. However, the overwhelming majority of the

*heterogamic copulations in which these displays culminate remain incomplete.*

*Functional Difficulties.*—Entomologists have long contended that the peculiarities of the sexual armatures might and occasionally do prevent interspecific crosses. The notion of a complete fit of a lock and key mechanism of male and female genitalia in its most exaggerated form is undoubtedly not correct, as pointed out by Dobzhansky<sup>12</sup> and other authors. However, mechanical difficulties do exist in most interspecific matings and reduce their efficiency as indicated by Sturtevant<sup>4</sup> and described in detail by Stalker<sup>6</sup> for cross matings between *D. virilis* and *D. americana*. Observations of the cross matings of *D. pseudoobscura* and *D. persimilis* fully confirm this. The following protocol may be added to those recorded above.

"July 24 (9:54 A.M.). 30 minutes. 3 *pseudoobscura* ♂ (6 days old), 4 *persimilis* ♀ (7 days old). During the first 7 minutes only one male is active. At least 16 incomplete copulations are counted during this period. In the next 10 minutes all 3 males are active, attempting to copulate with 2 of the females. I count 48 genital contacts during this 10-minute period and undoubtedly overlooked several others during this frenzy of activity. None of these 70 or more contacts leads to a completed copulation. All flies are quiescent during the final 6 minutes of the observation period. The females do not run away from the males, in fact they engage in 'invitation displays.' "

The conclusion to be drawn from these and other similar observations is inevitable. There must be some anatomical or physiological obstacle which prevents in most cases the completion of the interspecific copulations. Copulation is, of course, not entirely impossible, and if *pseudoobscura* ♂ stay sufficiently long with *persimilis* ♀ they will eventually inseminate most of them.

What the obstacle is that makes these interspecific matings so difficult has not yet been determined. A very careful study of the sexual armatures of males and females in the two species by Ferris and other workers has not yielded any apparent differences. There are obviously no "mechanical" barriers in the conventional meaning of the entomological literature. However, there may be invisible differences in the texture of the mucous membranes or in other physiological properties of the genital apparatus. Or else, the proper intromission of the phallus may require a high degree of receptivity ("coöperation") on part of the female. It is possible that the stimulation by the combined pre-copulatory display activities of the non-conspecific male is insufficient to produce the degree of receptivity in the female necessary for successful copulation. Observations gave the hard-to-prove impression that it was the female that was mainly responsible for the incompleteness of so many of the interspecific copulations. Further observations and experiments are required to solve this problem.

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## TWO TYPES OF HETEROCHROMATIN IN *DROSOPHILA NEBULOSA*

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*Introduction.*—Euchromatin and heterochromatin are the two principal components of plant and animal chromosomes. Heitz<sup>1</sup> was the first to make a study of the relative quantities and distribution of these components in the mitotic chromosomes of *Drosophila*. Working with *Drosophila tenebris*, he showed that in this species the heterochromatin is concentrated mostly in the sex chromosomes, making up the entire Y and half of the length of the X-chromosome which includes the centromere. The autosomes and the other half of the X-chromosome are formed mainly of euchromatin. In another work<sup>2</sup> the same author discussed the appearance of the heterochromatin in the salivary gland chromosomes. Here the heterochromatin forms the chromocenter and the bases of some of the chromosomes. Studying the salivary gland chromosomes of *D. virilis*<sup>3</sup> he concluded that the heterochromatin of this species is of two types, which he called, respectively,  $\alpha$ -heterochromatin and  $\beta$ -heterochromatin. In the salivary gland nuclei, the  $\alpha$ -heterochromatin forms a compact body, while

the  $\beta$  is more diffuse. While the  $\alpha$  type forms only a part of the chromocenter, the  $\beta$  type forms parts of this structure and the bases of the chromosomes.

Painter<sup>4</sup> working with salivary gland chromosomes of *D. melanogaster* considered the chromocenter: "an amorphous mass of chromatic material to which all chromosomes or arms are attached." Muller and Prokofjeva<sup>5</sup> found in the heterochromatic part of the X-chromosome of *D. melanogaster* that "the appearance of this region in salivary gland material indicates that its chromonema has essentially the same structure as that of the active region. . . ." Bauer<sup>6</sup> working in *Chironomus* and in some species of *Drosophila* concluded that heterochromatic regions of salivary gland chromosomes are composed of the same number of chromonemata as the euchromatic portions, the difference between them being due to the structure of the single chromomeres. The euchromatin is formed by small completely stained dots (euchromomeres) while the heterochromatin is formed of large dots stained on the periphery (heterochromomeres).

Muller, Gershenson and Prokofjeva-Belgovskaya<sup>7</sup> showed that relatively long sections of the mitotic X-chromosome of *D. melanogaster* are reduced each to a single band in the salivary chromosome. Hinton,<sup>8</sup> also working in *D. melanogaster*, compared the heterochromatin of chromosome II in the mitotic and the salivary chromosomes. He showed that a heterochromatic section constituting  $\frac{1}{8}$  of the left arm of the second chromosome in the mitotic metaphase is reduced to a single band in the salivary chromosome. Another region of about the same size, also in the second chromosome, gave rise to the remainder of the bulk of the heterochromatin of this salivary gland chromosome.

The present paper deals with the situation in *D. nebulosa*, in which there are two easily distinguishable types of heterochromatin. They have the same staining properties in the mitotic prophase and metaphase chromosomes, yet one of them is reduced in the salivaries to a few chromomeres only, while the other forms the bulk of the chromocenter. Since the two types of heterochromatin are confined to different chromosomes, the distinction between them is easy and more accurate than that in any other *Drosophila* species previously examined.

*Material and Method.*—*D. nebulosa* Sturtevant is the species used in the present work. It belongs to the *willistoni* group of the subgenus *Sophophora*. It has a wide distribution in the tropical Americas, from southern United States (Texas and Florida) to southern Brazil (São Paulo), including the West Indies.

Strains from Bertioga and Iporanga in the State of São Paulo, from Belem, State of Para, all in Brazil, and one strain from Del Rio, Texas, were used in this work. The last strain was kindly furnished by Prof. J. T. Patterson of the University of Texas.



All preparations were made by smearing the larval brain or salivary glands in acetic orcein.

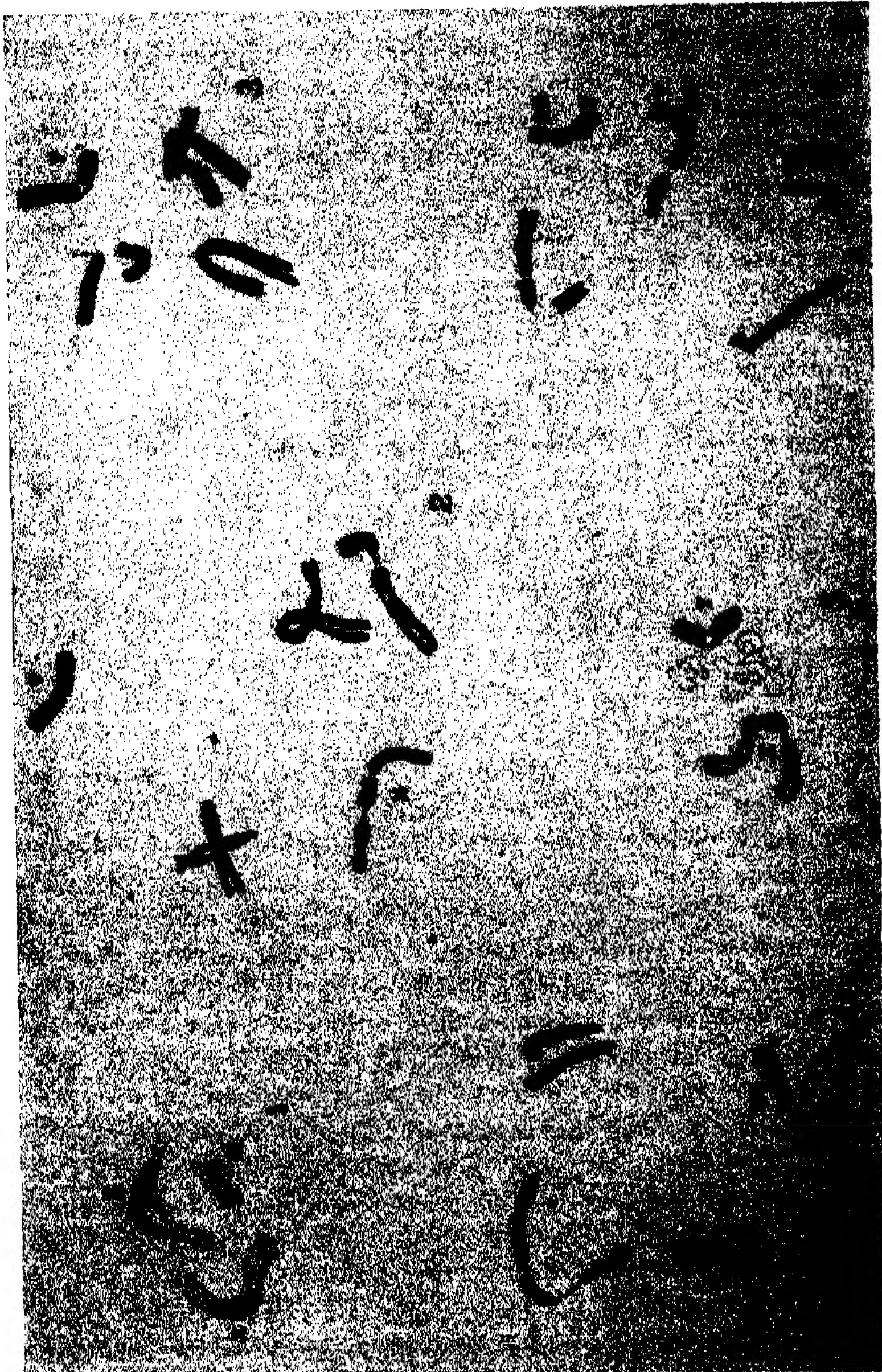
*Metaphase Chromosomes.*—A metaphase plate of a neuroblast of the larval ganglia of *D. nebulosa*, as well as of all other known species of the *willistoni* group, shows six chromosomes—two pairs of equal-armed V-shaped chromosomes and a pair of rods. The sex chromosomes are represented by one of the V-shaped pairs. In the female these two chromosomes are alike; in the male a slight difference between them, due to a greater compactness of the Y, can sometimes be noticed.

*Distribution of the Heterochromatin in the Mitotic Prophase Chromosomes.*—As the four V-shaped chromosomes are alike at metaphase the autosomes and the sex chromosomes cannot be distinguished. At the prophase the discrimination is possible because of the difference in the distribution of the eu- and heterochromatin. The Y-chromosome is easily distinguished from the other chromosomes because it is entirely heterochromatic and therefore stains more deeply (Figs. 2 and 5). The difference between the second chromosome and the X is much less obvious, because both chromosomes have approximately the same quantity and distribution of heterochromatin (see Fig. 1). The X-chromosome does have slightly more heterochromatin near the centromere than the second does, but the difference is not too striking. The rod-shaped third chromosome has a small piece of heterochromatin close to the centromere (Fig. 1).

*Salivary Gland Chromosomes.*—Although the chromosomes of the salivary gland cells of *D. nebulosa* are not very favorable for study, enough good preparations can be made. A well-smeared preparation shows a compact chromocenter from which radiate five long euchromatic strands. All five strands are equally stained in the females, but in the males two of them are stained less intensely. These two strands correspond to the two arms of the X-chromosome, which is haploid in the male.

By pressing the preparation one can individualize the chromosomes in such a way that the chromocenter breaks into its constituents, and each chromosome takes with itself the heterochromatin belonging to it. The breaking up of the chromocenter in the salivaries of *D. nebulosa* shows that almost all its material invariably goes with the second chromosome, leaving the X and the third chromosome with very little heterochromatin. The two arms of the second chromosome contain approximately equal pieces of heterochromatin.

The unequal distribution of the heterochromatin in the salivary gland chromosomes contrasts with the observation made on the heterochromatin in the mitotic prophase chromosomes, where the X-chromosome is provided with as much or more heterochromatin than the second, and where the third chromosome also has a small but easily noticeable heterochromatic section. This contrast is even more striking in the male. At mitotic



FIGURES 1-6

Figure 1—Early prophase in a neuroblast of a normal female of *Drosophila nebulosa*. Figures 2 and 5—Prophases in normal males. Figure 3—Prophase in Blade male. Figures 4 and 6—Prophases in *Echinus* males.



metaphase, the *Y*-chromosome differs from the *X* and the V-shaped autosomes only in its somewhat smaller size. At prophase, the *Y* is seen to be wholly heterochromatic while the *X* and the second have heterochromatic sections only in the vicinity of the centromeres. The heterochromatic section in the *X* is a little larger than that in the second. Finally, in the salivaries, the heterochromatin of the second chromosome makes up most of the chromocenter, while that of the *X* and the *Y* is reduced to only a few heterochromomeres.

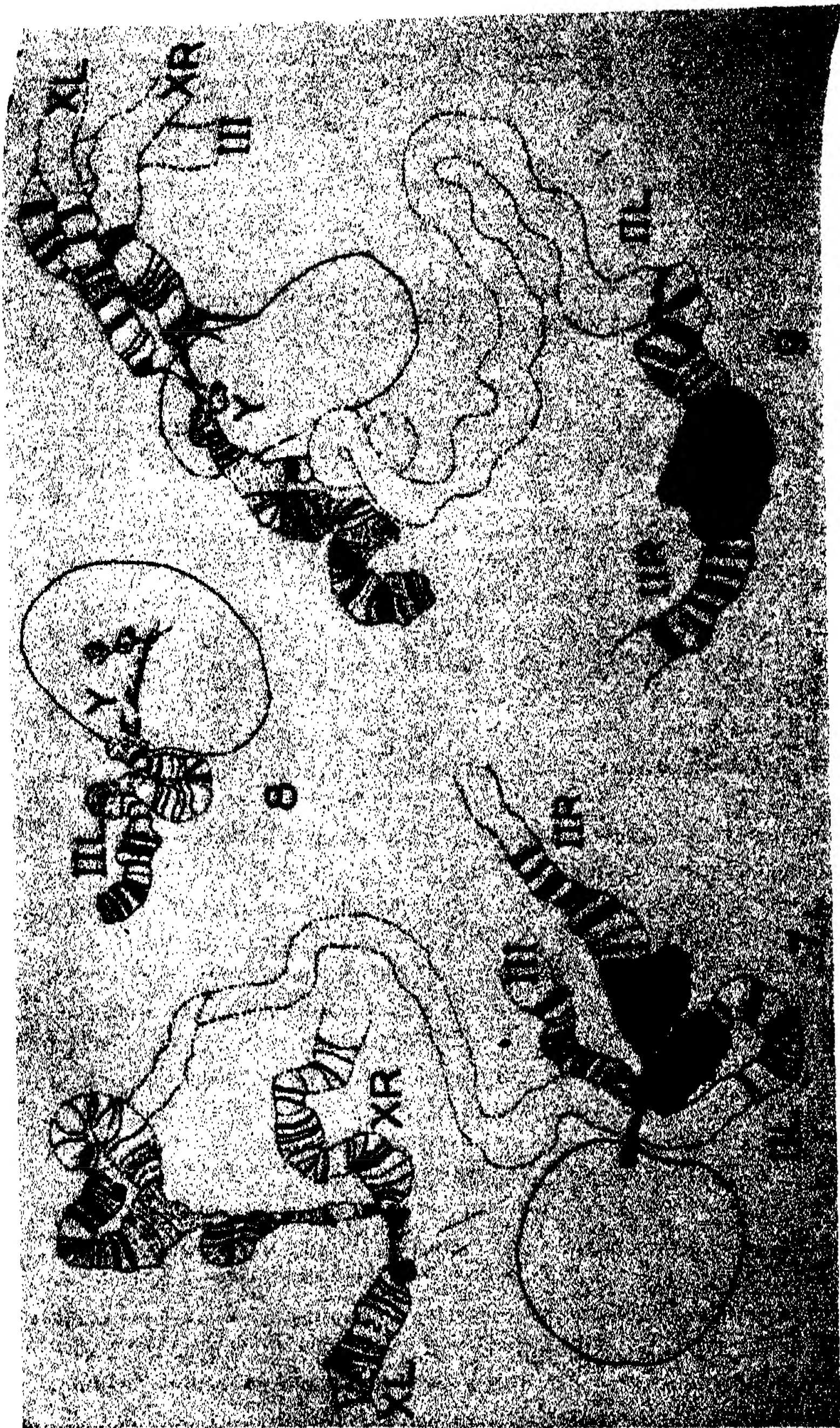
The proof of the above interpretation is afforded by the analysis of two reciprocal translocations involving parts of the *Y* and parts of the second chromosome.

*Translocation Blade.*— Among the mutations induced by x-rays in *D. nebulosa* males (4000 r), there was one which affected the shape of the wings. This mutation, which gives to the wing the sharp and narrow appearance called Blade, is transmitted from father to son, never appearing in females. Crosses between Blade males and normal females gave in  $F_1$  the following results: normal body and normal wings ♀, 339; minute body and normal wings ♂, 173; normal body and Blade wings ♂, 181.

The males with minute bodies and normal wings are sterile. This suggests that these males carry a chromosomal aberration.

The mitotic prophase of the Blade flies appears normal, indicating a reciprocal translocation involving only small pieces of the affected chromosomes. It can easily be seen in the salivary gland cells of male larvae that the left limb of the second chromosome makes a loop touching the chromocenter at a point about four-fifths of the length of that limb from the base. When the chromocenter is crushed by the pressure on the cover slip, a great portion of the chromocenter goes, as usual, with the second chromosome. However, the terminal portion of the left limb of the second chromosome remains attached to the very small part of the chromocenter which goes with the *X*-chromosome (Figs. 7 and 9). This portion may show a pairing with its homologue in the normal second chromosome, as can be seen in the figures mentioned above, or else pairing may not be attained, in which case a small unpaired section of the second chromosome is attached to the chromocentral part of the *X*, and the rest of the second chromosome is free with its terminal part being thinner than the rest.

Some individuals show an unpaired section of the terminal part of the left limb of the second chromosome attached to the chromocenter of the *X* (Fig. 8) and a normally paired second chromosome as well. These individuals evidently carry a duplication for the terminal part of II-L. Their origin is due to the translocated male forming some spermatozoa that carry a normal second chromosome as well as a section of another second chromosome attached to the *Y*-chromosome. However, another portion of the *Y*, which is translocated onto the basal portion of the second chromosome, is



FIGURES 7-9

Figures 7 and 9—Chromocenter and the bases of the chromosome strands in the Blade translocation. Figure 8—The duplicating fragment of the left limb of the second chromosome in the Blade translocation.

absent in these males. This probably accounts for the sterility of the non-Blade males with minute bodies mentioned above; these males lack a portion of the *Y*-chromosome.

*Translocation Echinus*.—Among the offspring of the x-ray irradiated males giving rise to Blade, there appeared another mutation called Echinus. This mutant has rough eyes, with some ommatidia larger than others and with the rows of the ommatidia disarranged. The Echinus eyes are also somewhat smaller than normal. Like Blade, the Echinus mutation is transmitted from father to son only.

Crosses of Echinus males to wild-type females gave: normal ♀, 485; normal ♂, 2; Echinus ♂, 349.

One of the two exceptional normal males observed in the  $F_1$  generation was mated to several females but produced no offspring. The other male died before it could be tested.

In this translocation the chromosome parts involved in the exchange are relatively long, and can be seen in the mitotic prophase chromosomes (Figs. 4 and 6) as well as in the salivary gland cells (Figs. 10, 11 and 12). In the prophase figures one can see that almost one-half of the heterochromatic *Y* and one-third of the right limb of the second chromosome are involved in the translocation (Figs. 4 and 6).

The salivary chromosome analysis shows that the translocation involves parts of II-R and the *Y*-chromosome. As in the case of Blade, the parts of II-R involved in the Echinus translocation are now associated with the chromocenter but most cells are paired with the normal II-R. Individualization of the chromosomes with the aid of fragmentation of the chromocenter shows that the bulk of the chromocenter goes with the second chromosome; the *Y* is represented by a few chromomeres, and the *X* and III have very little heterochromatin.

In most cells the base of the II-R forms a loop touching the chromocenter. A careful examination of this loop discloses few heterochromatin strands which attach an interstitial portion of the II-R to the chromocenter (Fig. 10). The heterochromatic strands must represent the *Y*-chromosome. In some cells, the sections of the second chromosome involved in the translocation remain unpaired with their homologues. Thus, figures 11 and 12 show the basal portion of the II-R extending from its heterochromatic part (on the right of both figures) to the translocation break (on the left). At that breakage point there is attached a group of heterochromomeres and heterochromatic strands which tend to associate with the heterochromatin of the *X*. These heterochromomeres represent, then, the part of the *Y*-chromosome translocated to the II-R.

*Discussion and Summary*.—Taking into account the results of the previous workers and the data presented in this paper, it can be asserted that there exist at least two types of heterochromatin. One type shows





FIGURES 10-12

Chromocenter and the second chromosome in the Echinus translocation.

relatively little reduction in the salivary gland cells in proportion to the euchromatin in the mitotic chromosomes. Another kind of heterochromatin, which is not distinguishable from the first by its staining properties in the mitotic prophase, is reduced in the salivary gland cells to only a few heterochromomeres. The question of whether or not the two types of heterochromatin found in *D. nebulosa* correspond to the  $\alpha$  and  $\beta$  types of Heitz<sup>3</sup> in *D. virilis* must be left open, because in this latter species it is difficult to localize these types of heterochromatin in the mitotic chromosomes and to compare them with the condition found in the salivary glands. It is necessary to point out that while Heitz<sup>3</sup> considered the  $\alpha$  heterochromatin of *D. virilis* devoid of chromomeres, the heterochromatin of the Y-chromosome of *D. nebulosa* which might seem to resemble his  $\alpha$  type is formed by typical Bauer's heterochromomeres.

The observations on the two types of heterochromatin found in *D. nebulosa* resemble most closely those of Muller, Gershenson and Prokofjeva-Belgovskaya<sup>7</sup> on the X-chromosome of *D. melanogaster* and those of Hinton<sup>8</sup> on the second chromosome of the same species.

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## GENES WHICH DIVIDE SPECIES OR PRODUCE HYBRID VIGOR

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New light has been shed by recent genetic investigations on the old question of the origin of species. It is becoming increasingly clear that a species consisting of a group of organisms similar in appearance and freely interbreeding, may in the course of time become subdivided into groups

which no longer interbreed and which, becoming increasingly different in details of structure or function, constitute genuinely distinct species. The question is how do the specific differences originate. What is it that prevents interbreeding between groups of individuals destined to become distinct species?

Several methods of origin of specific differences have been demonstrated which involve change in the number of the chromosomes or in their structure. Such are (1) autopolyploidy, doubling of the chromosome number without qualitative change, converting a diploid species into a tetraploid; (2) allopolyploidy, union of gametes from different species followed by doubling of the new chromosome aggregate, insuring fertility of the hybrid under selfing or close inbreeding, but preventing backcrossing with either parent species; (3) change in the structure of individual chromosomes, as by inversion, deletion or interchange of parts between different chromosome pairs, resulting in partial sterility.

All such changes act as barriers to interbreeding between groups of individuals differing in one or more of the ways mentioned.

Lamprecht<sup>1</sup> maintains that a species barrier may also arise by a gene mutation which involves no gross chromosome change but only the coming into existence of a new dominant gene, the recessive allele of which causes sterility in certain combinations. He regards this as a more important if not more common mode of origin of specific differences than changes in chromosome numbers or gross chromosome structure.

It will be easier to grasp Lamprecht's idea if we have in mind the principle of a "killer" gene demonstrated in *Paramecium* by Sonneborn.<sup>2</sup>

He showed that a dominant "killer" gene *K* is carried without harmful effects in one race of *Paramecium aurelia*, and in other races of the same species a recessive allele *k* of the killer gene is carried also without harmful effects, though it makes the race "sensitive" to the killer product of the killer race. So that, if the two races are brought together, a killer substance produced in the killer race will cause the death of all individuals of the other "sensitive" race exposed to the action of killer substance.

Thus, the two races are prevented from interbreeding as a probable consequence of a single gene mutation.

To understand how such a situation may have arisen, let us suppose that in a self-fertilizing species a new dominant potentially lethal gene (*K*) arises, perhaps in the seemingly "inert" chromatin, in one member of a synaptic pair. The material opposite *K* in synapsis becomes its recessive allele *k* and may become sensitized to *K*. The two now separate and pass into different gametes and zygotes, each harmless except in association with the other in a newly formed zygote. Gamete *K* will pass into a zygote *K*/—, and this will produce progeny, some *KK*, others *K*/—, and still others —/—, fully normal. Similarly, gamete *k* will pass into a



zygote  $k/-$ , and produce descendants  $kk$ ,  $k/-$  and fully normal  $-/-$ . Homozygous lines  $KK$  and  $kk$  may ultimately be established by chance isolations, but they will be unable to cross with each other because of a lethal barrier, since  $K$  potential killer +  $k$  sensitizer produces a zygote  $K/k$  in which active killer substance is produced.

The situation just outlined is not purely hypothetical. It is exactly realized in crosses between certain species of *Crepis* investigated by Hollingshead.<sup>3</sup> In crosses of *C. tectorum* with *C. capillaris* and two other *Crepis* species, hybrid offspring were produced which failed to develop beyond the cotyledon state. It is assumed by Hollingshead that plants of *C. tectorum* which produce interspecific hybrids, all of which perish, are homozygous for a potentially lethal gene, which they transmit in all their gametes. Let us call the lethal gene  $K$ , and an assumed recessive allele and sensitizer of  $K$  in *C. capillaris*,  $k$ . Then in  $F_1$  hybrids  $K$  (the potential lethal) combines with the sensitizer  $k$  to form a lethal  $K/k$  zygote.

Hollingshead was fortunate in finding other *C. tectorum* plants which in crosses with *C. capillaris* produced only 50 per cent of lethal seedlings, the other 50 per cent being viable and fully fertile in  $F_1$  and subsequent generations.

We may assume, as Hollingshead does, that such a *C. tectorum* plant was heterozygous for the lethal gene. Its constitution may be expressed as  $K/-$  (not  $K/k$ ), since no sensitizer action was shown.

The 50 per cent of viable  $F_1$  hybrids, would derive  $k$ , the sensitizer allele of  $K$ , from their *C. capillaris* parent, but this would be harmless in the absence of  $K$ . Their genetic formula would be  $k/-$  since they would carry only a single dose of  $k$ . But in later generations their descendants under selfing would become  $k/k$ ,  $k/-$  and  $-/-$ . In reality Hollingshead in her experiments found all *C. capillaris* plants tested to be homozygous  $k/k$  for the assumed recessive allele and sensitizer of  $K$ , since all gave the lethal reaction when combined with ordinary  $K$  gametes of *C. tectorum*.

Hollingshead found that in crosses with two other species of *Crepis*, no lethal action of the potentially lethal gene of *C. tectorum* was found, the  $F_1$  hybrids all being viable. It is obvious that such species do not carry the assumed sensitizer  $k$ . Their formula as regards the genetic locus occupied by  $K$  of *C. tectorum* and  $k$  of *C. capillaris* being  $-/-$ . This would correspond with the constitution of ancestral species of *Crepis* before the advent of the mutation  $K/k$ , as also with that of  $-/-$  derivatives of  $k/-$  zygotes.

In the light of the foregoing, let us now examine a case assumed by Lamprecht to fall in a category of species separating mutations.

Two species of cultivated beans, *Phaseolus vulgaris* and *P. multiflorus*, were found, when artificially crossed, to produce vigorous and fertile  $F_1$  hybrids. In  $F_2$  all characters, in which the two species differ, recombine freely in viable zygotes, with a single exception. The exception concerns a

gene *Epi* of *P. vulgaris*, and its allele *Hyp* of *P. multiflorus*. *Epi* is responsible for the epigeal position of the cotyledons in seedlings of the common bean germinated in soil; *Hyp* is responsible for the hypogeal position of the cotyledons in germinating seeds of *P. multiflorus*.  $F_1$  hybrids show an intermediate character.

When *P. vulgaris* is the mother plant in the cross, the  $F_1$  plant has *vulgaris* plasma. In that plasma gametogenesis is entirely normal, segregating into carriers of *Epi* and *Hyp*, respectively. The former enter into the production of fully fertile plants being in their native plasma, but the latter fail to develop further; for they are in foreign plasma which apparently does not give them the normal stimulus necessary to fruitful development.

In the reciprocal cross, in which the  $F_1$  plant derives its plasma from *P. multiflorus*, the *Hyp* gene produces fruitful offspring (being in its native plasma) but carriers of the *Epi* gene fail to develop further.

It thus appears that *Epi* and *Hyp* are both dominant species-specific alleles at a common genetic locus, occurring normally in different species.  $F_1$  hybrids show an intermediate character, thus demonstrating that both are present in an active state. As regards this gene pair,  $F_1$  is *Epi/Hyp* in formula. But each allele in its native plasma acts as a *sensitizer* of that plasma to the other allele, which thus becomes sterilized. In mother plants having *Epi* plasma, *Hyp* gametes fail to develop; and in mother plants having *Hyp* plasma, *Epi* gametes fail to develop.

It is possible that the puzzling phenomenon of hybrid vigor may find its explanation along similar lines. Jones,<sup>4</sup> has recently shown that heterosis may be found in the superior growth energy of a hybrid between two inbred lines of maize, related as mother and daughter strains, and differing from each other in a single gene pair, the dominant allele being found in the mother strain, the recessive in the daughter strain. On the evidence presented by Jones, it can be only the stimulating action resulting from the union of the dominant with the recessive allele of that single pair of genes which is accountable for the increased growth energy, superior to that of either parent strain.

Let us suppose that in the mother strain a new dominant gene *A* has made its appearance in the unorganized chromatin of a single chromosome, and that it has sensitized (after the manner of anaphylaxis) the chromatin which lay opposite that locus in the other member of the chromosome pair, thus creating a recessive allele, *a*.

The two alleles will pass into different gametes and may later become components of different homozygous strains, *AA* in the mother strain, *aa* in the mutant daughter strain. Each strain will maintain its distinctive morphological character, so far as this gene pair creates a distinction. Each also will manifest a characteristic growth energy. But when the two are

crossed, an increased growth energy is shown by the hybrid, a hybrid, be it remembered, as Jones clearly shows, in only a single gene pair,  $Aa$ .

This case is similar to that of the killer mutation of Sonneborn, except that the action induced in the dominant gene by its sensitized recessive, instead of being harmful is in this case beneficial.

Jones points out that in maize and other plants not all heterozygous unions of a dominant with its recessive allele result in increased growth energy. In the production commercially of hybrid corn, not all crosses of inbred lines result in hybrid vigor. In such cases no sensitizer action between a dominant and its recessive allele seems to have occurred. The alleles instead of being  $A$  and a sensitized recessive allele  $a$ , would seem to be  $A$  and an absence of a sensitized allele. In the cases reported by Jones as showing heterosis, the zygote may be assumed to be  $A/a$  in constitution. In cases not showing hybrid vigor, it may possibly be  $A/-$ , the  $-$  representing the condition of that locus in the chromatin prior to the origin of the  $A$  gene.

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## PRIME NUMBER OF OPERATORS IN SETS OF CONJUGATES

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Suppose that the order of the group  $G$  is  $p^m$ ,  $p$  being a prime number, and that each set of non-invariant conjugate operators of  $G$  contains  $p$  operators. Each of the non-invariant operators of  $G$  is then invariant under a subgroup of index  $p$  under  $G$  and this subgroup is therefore an invariant subgroup of  $G$  and includes the given  $p$  conjugate operators as well as the central of  $G$ . This central is contained in an Abelian invariant subgroup of  $G$  whose order is  $p$  times the order of this central and is itself contained in an invariant subgroup of  $G$  whose order is  $p^2$  times the order of the central of  $G$ . This subgroup  $H_1$  transforms its own operators in exactly the same way as these operators are transformed under  $G$  itself and contains the same central as  $G$  contains since each non-invariant operator of  $G$  is assumed to have exactly  $p$  conjugates under  $G$ .

The group of inner isomorphisms of  $G$  can be represented as an intransitive permutation group each of whose transitive constituents is of degree  $p$ . These constituents must also be of order  $p$  since the order of every operator of  $G$  is a power of  $p$ . The group of inner isomorphisms of  $G$  is therefore an Abelian group all of whose operators besides the identity are of order  $p$ . It will be proved that in this Abelian group of order  $p^n$ ,  $n$  is always an even number. In the given subgroup  $H_1$   $n$  is 2 and the commutator subgroup is of order  $p$  because this commutator subgroup is in the central of  $G$ , and if its order would exceed  $p$  then  $G$  would contain a set of more than  $p$  conjugate operators, which is contrary to the hypothesis. Since the group of inner isomorphisms of  $G$  is Abelian, the commutator subgroup of  $G$  is included in the central of  $G$ .

It was noted near the close of the first paragraph that the operators of  $H_1$  are transformed under  $H_1$  in the same ways as they are transformed under  $G$ . Hence the operators of  $G$  which are not also found in  $H_1$  include at least one operator  $s_1$  which is commutative with every operator of  $H_1$  and has its  $p$ th power in  $H_1$ . If the order of  $G$  exceeds  $p^2$  times the order of the central of  $G$  this will clearly be also true of the operators which are found in  $G$  but do not appear among the operators of a larger invariant subgroup of  $G$ . In particular, the subgroup  $H_1$  can be extended so as to obtain a group whose order is  $p^4$  times the order of the central of  $G$  and which is invariant under  $G$  and transforms its non-invariant operators in the same ways as these operators are transformed under  $G$  and involves the same central as  $G$  involves.

To prove that this extended group involves the same commutator subgroup as  $H_1$  involves we may assume that its commutator subgroup involves two independent generators  $t_1, t_2$  and prove that this leads to a contradiction. It was noted above that the commutator subgroup of  $G$  cannot involve any operator of order  $p^2$  since the commutators of  $G$  appear in its central. We may assume that the two operators  $s_1$  and  $s_2$  of  $H_1$  give rise to the commutator  $t_1$  and that the two operators  $s_3$  and  $s_4$  of the given subgroup  $H_2$  whose order is  $p^2$  times the order of  $H_1$  give rise to the commutator  $t_2$ . Moreover, it may be assumed that both of the operators  $s_3$  and  $s_4$  are commutative with each of the two operators  $s_1$  and  $s_2$ . It would then follow that the product  $s_1s_3$  would have more than  $p$  conjugates under  $G$ ; which is contrary to the hypothesis. Hence it results that *if in a group of order  $p^m$ ,  $p$  being a prime number, all the non-invariant operators have  $p$  conjugates the commutator subgroup is of order  $p$  and is in the central of  $G$ .*

To prove that  $n$ , the index of the power of  $p$  which is equal to the order of the group of inner isomorphisms of  $G$ , is always even it is only necessary to observe that in constructing  $G$  in the manner noted above, the order of the resulting group which transforms its operators in the same ways as the operators are transformed under  $G$  proceeds by  $p^2$  times the order of the



group already found whose operators have this property. For instance, the order of  $H_2$  is  $p^2$  times the order of  $H_1$  and the order of  $H_1$  is  $p^2$  times the order of the central of  $G$ . The group obtained by forming the direct product of  $G$  and any Abelian group clearly has the same group of inner isomorphisms as  $G$  has.

The fact that every set of non-invariant conjugate operators of a group is assumed to contain a prime number of operators by itself imposes a strong condition on a group but it does not necessarily restrict the order of the group to be a power of a single prime number. This results directly from the fact that if the order of a non-cyclic group is the product of two distinct prime numbers then the larger of these numbers diminished by unity is divisible by the smaller and each operator of the group besides the identity has a prime number of conjugates under the group. This is also true of the direct products of an arbitrary Abelian group and this non-cyclic group. A necessary and sufficient condition that the index of every proper subgroup of a group is a prime number is obviously that the order of the group is the product of two prime numbers.

If we assume that every set of non-invariant operators of  $G$  is composed of a prime number of operators but do not assume that the order of  $G$  is a power of a prime number then the operators which are commutative with a given non-invariant operator  $s$  of  $G$  constitute a subgroup of prime index under  $G$  which is not necessarily invariant under  $G$ . If this subgroup is assumed to be Abelian in every case then two such distinct subgroups will have a cross-cut which is in the central of  $G$  and hence is invariant under  $G$ . The quotient group of  $G$  with respect to this invariant subgroup has an order which is the product of two prime numbers. Hence it results that *when all the non-invariant operators of a group appear in sets of conjugates such that each set is composed of a prime number of operators and all the operators which are commutative with a given non-invariant operator constitute an Abelian group then the order of the central quotient group of the group is the product of two prime numbers.*

Since a group whose order is the product of two prime numbers always involves an invariant subgroup of prime index it has been proved that if all the non-invariant operators of a group appear in sets of conjugates which are such that the number of the operators in each set of conjugates is a prime number and if the total number of the operators which are commutative with a non-invariant operator always constitute an Abelian subgroup of prime index under the group then the group contains an invariant subgroup of prime index under the group. It may be noted that in the special system of non-cyclic groups whose orders are the products of two distinct prime numbers and in the direct products of such groups and arbitrary Abelian groups these conditions are satisfied. It should be emphasized that when each of two distinct subgroups is of prime index under



a given group then it is not possible for one of them to be contained in the other.

If the operators which are commutative with one of the non-invariant operators of one of the groups under consideration do not always constitute an Abelian group then the prime number of the conjugates for each of its sets of conjugate operators of its non-invariant operators must be the same and hence these groups were considered before in these PROCEEDINGS, 32, 53-56 (1946) under a somewhat more general title, which has much in common with the present article. This article aims to clarify some of the common points as well as to extend the types of the groups under consideration. The two articles together aim to consider fundamental questions of all the groups in which all the non-invariant operators appear in sets of conjugates such that each set involves a prime number of operators. It results that *the number of different primes involved in the same group cannot exceed two* and that when there are two such primes the larger diminished by unity is divisible by the smaller. The fact that there cannot be more than two such prime numbers in a given group seems to deserve special emphasis.

## COMPARISON OF UNION-PRESERVING AND CONTACT TRANSFORMATIONS\*

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1. *Union-Preserving and Contact Transformations.*—In our Bulletin paper of 1944, we studied transformations, in space, of curve-elements of order  $n$ , where  $n$  is 2 or more, into lineal-elements.<sup>1</sup> The general class of union-preserving transformations, where the point of the transformed lineal-element actually depends on the derivatives of order  $n$ , is defined by a single directrix equation

$$\Omega(X, Y, Z, x, y, z, y', z', \dots, y^{(n-2)}, z^{(n-2)}) = 0, \quad (U)$$

involving derivatives of order  $(n - 2)$ .

As an example, consider the problem of finding the locus of the center of spherical curvature of a twisted curve in space. There is deduced a union-preserving transformation, defined by the directrix equation

$$(X - x) + y'(Y - y) + z'(Z - z) = 0, \quad (E)$$

from curve-elements of third order into lineal-elements.

Sophus Lie studied transformations of surface-elements  $(x, y, z, p, q)$  into surface-elements. The general class of contact transformations in this classic theory is defined by the directrix equation

$$\Omega(X, Y, Z, x, y, z) = 0, \quad (L)$$

or by a pair or a triplet of such directrix equations. All the contact transformations of surface-elements of higher order into elements of same order are simply the extensions of these (as proved by Backlund).

If, and only if,  $n = 2$ , the directrix equation  $(U)$  does not contain any derivatives. The union-preserving transformations of curve-elements of second order into lineal-elements are defined by a single directrix equation which in this case is of the form  $(L)$ . Thus the directrix equation  $(L)$  defines a contact transformation  $\Gamma$  of surface-elements according to Lie, and a union-preserving transformation  $T$  from second-order curve-elements into lineal-elements according to our new theory.

In the present article, we shall give a comparison between the contact transformation  $\Gamma$  and the union-preserving transformation  $T$ , both of which are defined by the same directrix equation  $(L)$ . This phenomenon occurs in spaces of three or more dimensions. In the plane, we have already stated that  $\Gamma$  and  $T$  are identical<sup>2</sup> when there are no derivatives in  $\Omega$ .

2. *The Union-Preserving Transformation  $T$  from Curve-Elements of Second Order into Lineal-Elements.*—The point of the transformed lineal-element  $(X, Y, Z, Y', Z')$  is obtained by solving for  $(X, Y, Z)$ , the three equations<sup>3</sup>

$$\left. \begin{aligned} \Omega(X, Y, Z, x, y, z) &= 0, & \Omega_x + y'\Omega_y + z'\Omega_z &= 0, \\ \Omega_{xx} + y'^2\Omega_{yy} + z'^2\Omega_{zz} + 2y'z'\Omega_{yz} + 2z'\Omega_{xz} + 2y'\Omega_{xy} + y''\Omega_y + z''\Omega_z &= 0. \end{aligned} \right\} (1)$$

The direction is found by solving for  $(Y', Z')$ , the two extra equations

$$\left. \begin{aligned} \Omega_x + Y'\Omega_Y + Z'\Omega_Z &= 0, \\ (\Omega_{xx} + y'\Omega_{yx} + z'\Omega_{zx}) + Y'(\Omega_{xy} + y'\Omega_{yy} + z'\Omega_{zy}) + Z'(\Omega_{xz} + y'\Omega_{yz} + z'\Omega_{zz}) &= 0. \end{aligned} \right\} (2)$$

We consider briefly the transforms under our union-preserving correspondence  $T$ , of a lineal-element  $(x, y, z, y', z')$  and of a planar-element  $(x, y, z, p, q)$ .

In the first place, it is noticed that a single point  $(x, y, z)$  corresponds to a surface  $\Sigma$ , defined by the equation  $(L)$ . The lineal element  $(X, Y, Z, Y', Z')$  is that element on  $\Sigma$  which is on the edge of regression of the one-parameter family of surfaces which correspond by  $(L)$  to the points of the curve:  $y = y(x)$ ,  $z = z(x)$ .

A lineal-element  $(x, y, z, y', z')$  corresponds by (1) to a curve  $C$ , which is given by the first two of equations (1). This curve  $C$  is the locus of ultimate intersections of the two surfaces which correspond to two neighboring

points. Of course, the point of the transformed lineal-element  $(X, Y, Z, Y', Z')$  is on this curve  $C$ .

A planar-element  $(x, y, z, p, q)$  may be considered as consisting of the point  $(x, y, z)$  and the osculating plane of the second order curve-element. Thus  $(p, q, -1)$  are the direction numbers of the binormal, so that  $z' = p + qy'$  and  $z'' = qy''$ . In general, it is shown that a fixed planar element corresponds to the whole surface  $\Sigma$ . The complete correspondence is a single lineal-element at each point of  $\Sigma$ . That is, a planar-element corresponds to  $\infty^2$  lineal-elements, all tangent to the surface  $\Sigma$ .

By (1), it can be shown that *an arbitrary planar element can correspond to a single planar element if, and only if, each surface which is associated to any point  $(X, Y, Z)$  by (L), is a plane.*

A second union-preserving transformation can be defined by (L) from the  $(X, Y, Z)$ -space to the  $(x, y, z)$ -space. If this also converts a planar element into a planar element, then our correspondence must be a correlation.

3. *The Lie Contact Transformation  $\Gamma$ .*—The point of the transformed planar-element  $(X, Y, Z, P, Q)$  is found by solving for  $(X, Y, Z)$  the three equations<sup>3</sup>

$$\left. \begin{aligned} \Omega(X, Y, Z, x, y, z) &= 0, \\ \Omega_x + p\Omega_z &= 0, \quad \Omega_y + q\Omega_z = 0. \end{aligned} \right\} \quad (3)$$

The direction of the normal to the plane is obtained by solving for  $(P, Q)$  the two equations

$$\Omega_x + P\Omega_z = 0, \quad \Omega_y + Q\Omega_z = 0. \quad (4)$$

By (1) and (3), it is seen that the point of the transformed planar-element under the contact transformation  $\Gamma$  is on the curve  $C$  defined by the first two of equations (1). Of course, this is the characteristic point on the surface  $\Sigma$  of the two-parameter family of surfaces which correspond by (L) to the points of the surface:  $z = z(x, y)$ .

This characteristic point is different, in general, from the point of the transformed lineal-element under our union-preserving transformation  $T$ . *The two transformations  $T$  and  $\Gamma$  are effectively identical if, and only if, each surface which is associated to any point  $(X, Y, Z)$  by (L), is a plane.*

Let  $T'$  denote the union-preserving transformation which by (L) carries any second order curve-element  $(X, Y, Z, Y', Z', Y'', Z'')$  into a lineal-element  $(x, y, z, y', z')$ . In general, this is not the inverse of the original union-preserving transformation  $T$ . Also let  $\Gamma'$  be the inverse of the contact transformation  $\Gamma$ .

*The union-preserving transformations  $T$  and  $T'$  will coincide with the contact correspondences  $\Gamma$  and  $\Gamma'$ , respectively, only for the case of a correlation.*

We shall illustrate our general theory with some noteworthy examples.

4. *Examples.*—*Polarity* with respect to the sphere:  $x^2 + y^2 + z^2 = a^2$  is given by the directrix equation

$$xX + yY + zZ = a^2. \quad (5)$$

In this case, it is shown easily that not only  $T$  and  $\Gamma$ , but also  $T'$  and  $\Gamma'$ , are identical.

Next consider the directrix equation

$$(X - x)^2 + (Y - y)^2 + (Z - z)^2 = a^2. \quad (6)$$

This carries each point  $(x, y, z)$  into a sphere with radius  $a$  and with center at the original point.

The contact transformation  $\Gamma$  defined by (6) is known as *dilatation*. It moves each planar-element in a direction perpendicular to itself through a constant distance  $a$ . Any surface is turned into a parallel surface and we have the theorems of Gauss.

But the same simple equation (6) defines a new transformation  $T$  according to our theory which we term *quasi-dilatation*.

We thus obtain a new theory of quasi-parallel curves in space by means of the union-preserving transformation  $T$  whose directrix equation is (6).

This correspondence  $T$  is given by the equations

$$\left. \begin{aligned} (X - x)^2 + (Y - y)^2 + (Z - z)^2 &= a^2, \\ (X - x) + y'(Y - y) + z'(Z - z) &= 0, & y''(Y - y) + \\ & z''(Z - z) = 1 + y'^2 + z'^2, \\ (X - x) + Y'(Y - y) + Z'(Z - z) &= 0, & 1 + y'Y' + z'Z' = 0. \end{aligned} \right\} \quad (7)$$

This transformation  $T$  is constructed geometrically in the following manner. Draw the circle of curvature to any second order curve-element. Let  $B$  be the straight line through the center of this circle and orthogonal to the osculating plane. The corresponding points  $(X, Y, Z)$  are the intersections of this line  $B$  and the sphere (6). The directions  $(Y', Z')$  are perpendicular to the radius vector connecting the corresponding points  $(x, y, z)$  and  $(X, Y, Z)$  and the tangent direction of the original curve-element. Thus these three directions are mutually orthogonal.

Under our new theory of quasi-parallel curves, we find that *the quasi-parallel of a helix is a helix*. The quasi-parallel of a plane curve is on the cylinder with generators orthogonal to the plane of the original curve and passing through its evolute. In general, the new quasi-parallel of a plane curve is gauche.

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<sup>1</sup> "Union-Preserving Transformations of Space," *Bull. Am. Math. Soc.*, 50, 98-107 (1944).

<sup>2</sup> "Union-Preserving Transformations of Differential Elements," these PROCEEDINGS, 29, 271-275 (1943). Also "A Generalized Theory of Contact Transformations," *Revista de matematicas del Universidad de Tucuman (Argentina)*, 4, 81-90 (1944). A typical ex-

ample in this theory is Huyghen's transformation from any curve to its evolute. Our whole theory may be regarded as a natural extension of Huygen's discussion of evolutes, involutes and wave propagation; and optical aspects will be considered elsewhere.

\* In both the Lie theory and the new theory of the present paper, it is of course assumed that the directrix equation actually represents a triple infinity of surfaces in the  $(x, y, z)$  space. Hence certain functional determinants do not vanish.

## AN ERGODIC THEOREM

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The purpose of this note is to state and sketch the proof of a theorem of the ergodic type (Theorem 1) and to discuss and clarify the relations among Theorem 1 and the results of Birkhoff-Khintchine, Hopf and Hurewicz. It turns out that Theorem 1 (or, rather, its easy consequence, Theorem 6) includes as a special case the hitherto most inclusive result (namely the theorem of Hurewicz, which appears below as Theorem 7). The extent to which Theorem 6 is a generalization of the theorem of Hurewicz is exactly the same as the extent to which Hopf's result is a generalization of Birkhoff's. Both the statement and the proof of Theorem 1 are more natural, and in several details more simple, than those of Theorem 7. In section 5 the important question of the deducibility of Theorem 1 from the known results on measure preserving transformations is treated.

1. Let  $X$  be a measure space which is the union of countably many measurable sets of finite measure, and let  $T$  be a one to one transformation of  $X$  onto itself. The transformation  $T$  is *measurable* if both  $T$  and  $T^{-1}$  send measurable sets into measurable sets; it is *non-singular* if both  $T$  and  $T^{-1}$  send sets of measure zero into sets of measure zero. If  $T$  is measurable and non-singular then the Radon-Nikodym theorem yields the existence of a measurable function  $\omega_n(x)$  (which may be assumed to be everywhere positive) such that for every measurable set  $E$ ,  $m(T^n E) = \int_E \omega_n(x) dm(x)$ . For any real valued function  $q(x)$  and any positive integer  $n$  the notation  $q^n(x)$  will be used for the sum  $q^n(x) = \sum_{i=0}^{n-1} q(T^i x) \omega_i(x)$ .

**THEOREM 1.** *If  $T$  is measurable and non-singular,  $f(x)$  is integrable,  $g(x)$  is non-negative, measurable, and such that  $\lim_{n \rightarrow \infty} g^n(x) = \infty$  almost everywhere, then  $f^n(x)/g^n(x)$  converges almost everywhere to a finite limit.*

The proof of Theorem 1 appears in section 4.

Observe that  $f^n(x)/g^n(x)$  may be considered as the ratio of two expres-



sions of the form  $(\sum_{i=0}^{n-1} q(T^i x) \omega_i(x)) / (\sum_{i=0}^{n-1} \omega_i(x))$ . Such an expression is the average of the first  $n$  terms of the sequence  $\{q(T^i x)\}$  weighted by the first  $n$  terms of the sequence of densities  $\{\omega_i(x)\}$ . If it were permissible to let  $g(x)$  be identically equal to 1 (as it is, under certain conditions discussed below) then, for this  $g$ ,  $f^n/g^n$  itself would be a weighted average.

If  $T$  is measure preserving then  $\omega_i(x) = 1$  for every  $i$  and  $x$ , and for  $g(x) = 1$ ,  $g^n(x) = n$ . Consequently in this most important special case Theorem 1 reduces to the classical ergodic theorem.

**THEOREM 2** (Birkhoff<sup>1</sup>-Khinchine<sup>2</sup>). *If  $T$  is measure preserving and  $f(x)$  is integrable then  $(\sum_{i=0}^{n-1} f(T^i x))/n$  converges almost everywhere to a finite limit.*

2. The transformation  $T$  is *incompressible* if for every measurable set  $E$ ,  $TE \subseteq E$  implies  $m(E - TE) = 0$ . The function  $g(x)$  is *invariantly positive* if it is non-negative, measurable, and such that there is no invariant set of positive measure on which it vanishes. Observe that if  $g$  is measurable and almost everywhere positive (in particular if  $g(x) = 1$ ) then  $g$  is *a fortiori* invariantly positive.

**THEOREM 3.** *If  $T$  is measurable and non-singular,  $g(x)$  is non-negative and measurable, and  $\lim_{n \rightarrow \infty} g^n(x) = \infty$  almost everywhere, then  $g$  is invariantly positive. Conversely if  $T$  is measurable, non-singular, and incompressible, and if  $g$  is invariantly positive, then  $\lim_{n \rightarrow \infty} g^n(x) = \infty$  almost everywhere.*

*Proof.* If  $\lim_{n \rightarrow \infty} g^n(x) = \infty$  almost everywhere and  $A$  is a measurable invariant set such that for  $x \in A$ ,  $g(x) = 0$ , then for  $x \in A$ ,  $\lim_{n \rightarrow \infty} g^n(x) = 0$ . It follows that  $m(A) = 0$ , so that  $g$  is invariantly positive.

Suppose, conversely, that  $g$  is invariantly positive and define  $\mu(E) = \int_E g(x) dm(x)$ . Let  $k$  be any positive number and let  $E$  be any measurable set of finite measure on which  $\sum_{i=0}^{\infty} (T^i x) \omega_i(x) < k$ . Then  $\infty > km(E) \geq \int_E \sum_{i=0}^{\infty} g(T^i x) \omega_i(x) dm(x) = \sum_{i=0}^{\infty} \int_{T^i E} g(x) dm(x) = \sum_{i=0}^{\infty} \mu(T^i E)$ . If  $F = \bigcup_{n=0}^{\infty} T^n E$  then, since  $TF \subseteq F$  and  $T$  is incompressible, it follows that the sets  $F, TF, T^2 F, \dots$  are all equal except for a set of  $m$  measure zero and therefore except for a set of  $\mu$  measure zero. Hence

$$\mu(F) = \mu(\bigcap_{k=0}^{\infty} T^k F) = \mu(\bigcap_{k=0}^{\infty} \bigcup_{i=k}^{\infty} T^i E) \leq \sum_{i=k}^{\infty} \mu(T^i E),$$

and it follows that  $\mu(F) = 0$ . The incompressibility of  $T$  together with its non-singularity implies that the invariant set  $F^* = \bigcup_{n=0}^{\infty} T^n F$  differs from  $F$  on at most a set of measure zero, whence

$$0 = \mu(F) = \int_F g dm = \int_{F^*} g dm.$$

Since  $g$  is invariantly positive, it follows that  $m(E) \leq m(F) \leq m(F^*) = 0$ . Since, by the hypothesis on the space  $X$ , the set of points  $x$  at which  $\lim_{n \rightarrow \infty} g(x) < \infty$  is the union of countably many sets such as  $E$ , the proof of the theorem is complete.

Theorems 1 and 3 together imply the following two results.

**THEOREM 4.** *If  $T$  is measurable, non-singular, and incompressible,  $f(x)$  is integrable, and  $g(x)$  is invariantly positive, then  $f^n(x)/g^n(x)$  converges almost everywhere to a finite limit.*

**THEOREM 5 (Hopf<sup>3</sup>).** *If  $T$  is measure preserving and incompressible,  $f(x)$  is integrable, and  $g(x)$  is invariantly positive, then  $(\sum_{i=0}^{n-1} f(T^i x))/(\sum_{i=0}^{n-1} g(T^i x))$  converges almost everywhere to a finite limit.*

Hopf himself stated this result only under the condition that  $g(x)$  is integrable and almost everywhere positive. The present generality is sometimes technically useful.

3. The results of section 2 have applications even to the possibly singular case.

**THEOREM 6.** *If  $T$  is measurable and incompressible,  $f(x)$  is integrable, and  $g(x)$  is measurable and almost everywhere positive, and if  $h_n(x)$  is defined, by means of the Radon-Nikodym theorem, by the relation  $\sum_{i=0}^{n-1} \int_{T^i E} f(x) d\bar{m}(x) = \int_E h_n(x) d\mu^n(x)$ , where  $\mu^n(E) = \sum_{i=0}^{n-1} \int_{T^i E} g(x) d\bar{m}(x)$ , then  $h_n(x)$  converges almost everywhere to a finite limit.*

*Proof.* Assume first that  $m(X) < \infty$ . If  $\mu^n(E) = 0$  then, since  $g$  is positive,  $m(T^i E) = 0$  and therefore  $\sum_{i=0}^{n-1} \int_{T^i E} f(x) d\bar{m}(x) = 0$ , so that the Radon-Nikodym theorem may indeed be applied. Let  $\{c_n: n = 0, \pm 1, \pm 2, \dots\}$  be a sequence of positive constants whose sum is 1 and write  $\bar{m}(E) = \sum_{n=-\infty}^{\infty} c_n m(T^n E)$ . With respect to the finite measure  $\bar{m}$  the transformation  $T$  is non-singular and incompressible. To prove the latter statement, observe first that  $m$  and  $\bar{m}$  are identical on invariant sets. If  $E$  is a measurable set for which  $TE \subseteq E$  then  $T^{n+1}E \subseteq T^n E$ , so that

$$\bar{m}(E - TE) \leq \bar{m}(\bigcup_{n=-\infty}^{\infty} (T^n E - T^{n+1} E)) = m(\bigcup_{n=-\infty}^{\infty} (T^n E - T^{n+1} E)) = 0,$$

and therefore  $T$  is indeed incompressible with respect to  $\bar{m}$ . Since  $\bar{m}(E) = 0$  implies  $m(E) = 0$ , a non-negative measurable function  $p(x)$  may be found so that for every measurable set  $E$ ,  $m(E) = \int_E p(x) d\bar{m}(x)$ ; the fact that  $m$  and  $\bar{m}$  are identical on invariant sets implies that  $p(x)$  is invariantly positive with respect to  $\bar{m}$ . Since, finally,  $\int_X f(x) d\bar{m}(x) = \int_X f(x) p(x) d\bar{m}(x)$ , it follows that  $f(x)p(x)$  is integrable with respect to  $\bar{m}$ , and consequently that Theorem 4 may be applied to the transformation  $T$ , the measure  $\bar{m}$ , the integrable function  $\bar{f}(x) = f(x)p(x)$ , and the invariantly positive function  $\bar{g}(x) = g(x)p(x)$ . The conclusion is that  $\bar{f}^n/\bar{g}^n$  converges to a finite limit almost everywhere with respect to  $\bar{m}$  and hence almost everywhere with respect to  $m$ . The proof of Theorem 6 will be completed by calculating  $h_n$  and observing that it is equal to  $\bar{f}^n/\bar{g}^n$  almost everywhere. Since

$$\begin{aligned} \mu^n(E) &= \sum_{i=0}^{n-1} \int_{T^i E} g(x) d\bar{m}(x) = \sum_{i=0}^{n-1} \int_{T^i E} \bar{g}(x) d\bar{m}(x) \\ &= \sum_{i=0}^{n-1} \int_E \bar{g}(T^i x) \omega_i(x) d\bar{m}(x) = \int_E \bar{g}^n(x) d\bar{m}(x), \end{aligned}$$

it follows that

$$\int_E f^n(x) d\bar{m}(x) = \sum_{i=0}^{n-1} \int_E f(T^i x) \omega_i(x) d\bar{m}(x) = \sum_{i=0}^{n-1} \int_{T^i E} f(x) d\bar{m}(x) = \sum_{i=0}^{n-1} \int_{T^i E} f(x) dm(x) = \int_E h_n(x) d\mu^n(x) = \int_E h_n(x) \bar{g}^n(x) d\bar{m}(x);$$

the equality of the first and last members of the last written chain of equations, for all measurable sets  $E$ , implies the desired result.

If  $m(X) = \infty$ , let  $m'$  be a finite measure whose vanishing is equivalent to that of  $m$ . (The existence of such an  $m'$  is a consequence of the assumption that  $X$  is the union of countably many sets of finite measure.) Then  $m(E) = \int_E k(x) dm'(x)$  with a positive measurable function  $k(x)$ ; write  $f' = kf$ ,  $g' = kg$ , and apply the result just proved to  $f'$ ,  $g'$ ,  $m'$ . The fact that for any measurable set  $E$ ,  $\int_E f' dm' = \int_E f dm$  and  $\int_E g' dm' = \int_E g dm$  concludes the proof of Theorem 6.

If  $g(x) = 1$ , Theorem 6 may be stated as follows.

**THEOREM 7 (Hurewicz<sup>4</sup>).** *If  $T$  is measurable and incompressible and  $f(x)$  is integrable, and if  $h_n(x)$  is defined by the relation  $\sum_{i=0}^{n-1} \int_{T^i E} f(x) dm(x) = \int_E h_n(x) dm^n(x)$ , where  $m^n(E) = \sum_{i=0}^{n-1} m(T^i E)$ , then  $h_n(x)$  converges almost everywhere to a finite limit.*

4. In this section the proof of Theorem 1 is outlined. Since the proof uses no essentially novel methods and makes some very minor simplifications only, as compared with the corresponding proofs of Khintchine, Hopf and Hurewicz, a more than customarily condensed telegraphic style will be employed.

(A) Since  $\int_E \omega_{i+j}(x) dm(x) = m(T^{i+j}E) = \int_{T^j E} \omega_i(x) dm(x) = \int_E \omega_i \times (T^j x) \omega_j(x) dm(x)$ , the relations  $\omega_{i+j}(x) = \omega_i(T^j x) \omega_j(x)$  may be assumed to hold for every  $x$ . It follows that for  $1 \leq i < j = 1, 2, 3, \dots$ ,  $q^j(x) = q^i(x) + \omega_i(x) q^{j-i}(T^i x)$  for every real valued function  $q(x)$ .

(B) If  $q(x)$  is measurable and either its positive or its negative part is integrable (so that  $\int_E q(x) dm(x)$  is defined, although possibly infinite, for every measurable set  $E$ ) and if  $E = \bigcup_{n=1}^{\infty} \{x: q^n(x) \geq 0\}$  then  $\int_E q(x) dm(x) \geq 0$ . For if  $E_j = \{x: q^j(x) < 0, 1 \leq i < j; q^i(x) \geq 0\}$ , then for  $1 \leq i < j$  and for any  $x \in E_j$ ,  $q^{j-i}(T^i x) = (q^j(x) - q^i(x))/\omega_i(x) \geq q^j(x)/\omega_i(x) \geq 0$ , so that  $T^i E_j \subseteq \bigcup_{k=1}^{j-1} E_k$ . For any positive integer  $n$  write  $F_n = E_n$  and, by induction backward,  $F_{n-k} = E_{n-k}(X - \bigcup_{j=n-k+1}^n \bigcup_{i=0}^{j-1} T^i F_j)$ ; it follows that  $F_j \subseteq E_j$ ,  $j = 1, \dots, n$ ,  $\bigcup_{k=1}^n E_k = \bigcup_{j=1}^n \bigcup_{i=0}^{j-1} T^i F_j$ , and the  $n(n+1)/2$  sets in the last written double union are pairwise disjoint. Hence

$$\int_{\bigcup_{k=1}^n E_k} q(x) dm(x) = \sum_{j=1}^n \sum_{i=0}^{j-1} \int_{T^i F_j} q(x) dm(x) = \sum_{j=1}^n \sum_{i=0}^{j-1} \int_{F_j} q(T^i x) \omega_i(x) dm(x) = \sum_{j=1}^n \int_{F_j} q^j(x) dm(x) \geq 0.$$

(C) If for any real number  $c$ ,  $E^+(c) = \bigcup_{n=1}^{\infty} \{x: f^n(x) \geq c g^n(x)\}$  and  $E_-(c) = \bigcup_{n=1}^{\infty} \{x: f^n(x) \leq c g^n(x)\}$  then it follows by applying (B) first to

$q(x) = f(x) - cg(x)$  and then to  $q(x) = cg(x) - f(x)$ , that  $\int_{E^*(c)} f dm \geq c \int_{E^*(c)} g dm$  and  $\int_{E_*(c)} f dm \leq c \int_{E_*(c)} g dm$ . It follows also from these inequalities that, although  $g$  was not assumed to be integrable, its integrals over  $E^*(c)$  and  $E_*(c)$  are finite.

(D) If  $\int_X |f(x)| dm(x) = M < \infty$  then for  $c > 0$ ,  $\int_{E^*(c)} g dm$  and  $\int_{E_*(-c)} g dm$  are both  $\leq M/c$ , by (C). If  $F$  is the set of those points  $x$  for which the sequence  $\{f^n(x)/g^n(x)\}$  is not bounded then it follows from these inequalities and the relation  $F = \bigcap_{c>0} E^*(c) \cup \bigcap_{c>0} E_*(c)$  that  $\int_F g(x) dm(x) = 0$ . Hence  $g(x) = 0$  for almost every  $x$  belonging to the invariant set  $F$ ; Theorem 3 implies that  $m(F) = 0$ , i.e., that  $\{f^n/g^n\}$  is bounded almost everywhere.

(E) If  $h_*(x)$  and  $h^*(x)$  are, respectively, the limit superior and the limit inferior of  $f^n(x)/g^n(x)$ , then the relation  $f^n(Tx)/g^n(Tx) = (f^{n+1}(x) - f(x))/(g^{n+1}(x) - g(x))$  (proved by (A)), together with the assumption  $\lim_{n \rightarrow \infty} g(x) = \infty$  almost everywhere, implies that  $h^*(x)$  and  $h_*(x)$  are almost everywhere invariant under  $T$ .

(F) If  $M_{rs} = \{x: h_*(x) < r < s < h^*(x)\}$ , then  $m(M_{rs}) = 0$ . For, since (by (E))  $M_{rs}$  is invariant under  $T$ , the inequalities in (C) remain valid if  $M_{rs}$  is considered to be the whole space. Since  $M_{rs} \subseteq E_*(r)E^*(s)$ , it follows that  $s \int_{M_{rs}} g dm \leq \int_{M_{rs}} f dm \leq r \int_{M_{rs}} g dm$ ; since (also by (C))  $\int_{M_{rs}} g dm < \infty$ , the assumption  $r < s$  implies that  $\int_{M_{rs}} g dm = 0$ . The invariance of  $M_{rs}$  and Theorem 3 show that  $m(M_{rs}) = 0$ .

Since  $\{x: h_*(x) < h^*(x)\} = \bigcup_{r,s} M_{rs}$ , where the union is extended over all pairs of rational numbers, the conclusion of Theorem 1 follows from (F).

The proof shows also that the limit function  $h(x)$  is invariant under  $T$  (and, of course, measurable). A straightforward adaptation of standard methods can be used to show also that the product  $h(x)g(x)$  is integrable, and that for every invariant measurable set  $E$  for which  $\int_E g dm < \infty$ , the identity  $\int_E f dm = \int_E h g dm$  holds.

5. If  $T$  is non-singular and if there exists a measure  $m^*$  invariant under  $T$ , such that  $m^*(E) = 0$  if and only if  $m(E) = 0$ , and such that the space  $X$  is the union of countably many sets of finite  $m^*$  measure, then, at least in the incompressible case, Theorem 1 is an easy consequence of Hopf's theorem. (Whether or not such an  $m^*$  always exists is still an open question.<sup>6</sup>) The proof of this statement is similar to, but much simpler than, the proof of Theorem 6.

Under the conditions described, there exists a function  $p(x)$ , measurable and almost everywhere positive, such that for every measurable set  $E$ ,  $m(E) = \int_E p(x) dm^*(x)$ . Since  $\int_X f(x) dm(x) = \int_X f(x) p(x) dm^*(x)$ , it follows that  $f(x)p(x)$  is integrable with respect to  $m^*$  and consequently (assuming that  $T$  is incompressible) Theorem 5 may be applied to the transformation  $T$ , the measure  $m^*$ , the integrable function  $f^*(x) = f(x)p(x)$ ,

and the invariantly positive function  $g^*(x) = g(x)p(x)$ . The conclusion is that  $\sum_{i=0}^{n-1} f^*(T^i x) / \sum_{i=0}^{n-1} g^*(T^i x)$  converges to a finite limit almost everywhere with respect to  $m^*$  and hence almost everywhere with respect to  $m$ . Since, however,

$$\begin{aligned} \int_E \omega_n(x) p(x) dm^*(x) &= \int_E \omega_n(x) dm(x) = m(T^n E) \\ &= \int_{T^n E} p(x) dm^*(x) = \int_E p(T^n x) dm^*(x), \end{aligned}$$

it follows that  $p(T^n x) = \omega_n(x)p(x)$  almost everywhere. If the indicated substitution is made in the expressions whose convergence Hopf's theorem asserts, the asserted result follows.<sup>6</sup>

<sup>1</sup> Proof of a recurrence theorem for strongly transitive systems and proof of the ergodic theorem, these *PROCEEDINGS*, 17, 650 (1931).

<sup>2</sup> Zu Birkhoff's "Lösung des Ergodenproblems," *Mathematische Annalen*, 107, 485 (1932).

<sup>3</sup> *Ergodentheorie*, Berlin, 1937, p. 49.

<sup>4</sup> "Ergodic Theorem without Invariant Measure," *Ann. Math.*, 45, 195 (1944).

<sup>5</sup> This problem will be treated and necessary and sufficient conditions for the existence of such an  $m^*$  will be derived in a forthcoming paper, "Invariant Measures," submitted for publication to *Ann. Math.*

<sup>6</sup> I wish to express my thanks to J. C. Oxtoby for his critical reading of the manuscript of this paper and for making several valuable suggestions, and to G. W. Mackey, who called my attention to the fact that by methods similar to those used in this paper it is easy to deduce Theorem 6 from Theorem 7. This shows that the generalization of Hurewicz's theorem in the direction of Hopf's is in reality no generalization at all.





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## REVERSE-MUTATION AND ADAPTATION IN LEUCINELESS NEUROSPORA

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Mutations affecting specific steps in biochemical syntheses have been produced in *Neurospora crassa* by x-rays and ultra-violet radiation.<sup>1</sup> In crosses with wild-type molds, these mutations segregate in a Mendelian fashion and hence involve the alteration of single determinant factors. Since many of the mutants seem to completely lack a specific synthetic ability it was not certain whether they involved small chromosomal aberrations such as deficiencies, or the inactivation of genes which still self-duplicate. Up to the present time no demonstration of back-mutation, which would afford indirect evidence on this question, has been made for any of the biochemical mutants of *Neurospora*. On the basis of the evidence presented below we conclude that reverse-mutation to the wild-type allele does occur.

Strain 33757, which originated from a culture treated with ultra-violet light, is unable to synthesize the amino acid, leucine, an inability which has been shown by Regnery<sup>2</sup> to be caused by a difference from wild type in a single factor. In the course of the development of an assay method for leucine by the use of strain 33757 it was noted that the weights of some cultures were unusually high and did not bear the typical relationship to leucine in the medium which characterizes the growth of this mutant strain. The phenomenon was called adaptation.<sup>3</sup> Genetic and physiological studies have been made on three independently adapted strains of 33757.<sup>4</sup> Adapted strains  $\alpha$  and  $\beta$  were derived as follows from a leucineless albino-marked stock of mating type A (33757-4637-A). Conidia were inoculated into flasks containing 0.25 mg. l (+) leucine per 50-ml. medium and were incubated at 30°C. for 8½ days. Samples were taken from two cultures which subsequently proved to have unusually high weights, and inoculated into minimal medium. From this time, adapted cultures  $\alpha$  and  $\beta$  were

subcultured on minimal medium where they grew like wild type. The third adapted strain,  $\gamma$ , was obtained from a salmon-colored leucineless stock of mating type  $a$  (33757- $a$ ) which was heavily inoculated into minimal medium. In one instance growth was observed and, upon subculturing, this adapted strain was prototrophic<sup>b</sup> and grew like wild type.

It should be emphasized that marker genes, for color and for mating type, were not modified in the course of adaptation. Furthermore, among more than 150 adaptations of the leucineless albino stock which we have observed in our studies, none have shown pigmentation. Consequently it is concluded that the adapted strains did not arise through contamination.

*Genetic Studies.*—In order to test the presumption that adaptation involves a genic mutation, the adapted cultures were first crossed with a leucineless strain of the opposite mating type. Asci were dissected and the spores allowed to germinate on medium containing leucine. The resulting strains were then tested for their ability to grow on minimal medium. The results are given in table 1.

TABLE 1

CHARACTERISTICS OF  $f_1$  CULTURES OBTAINED BY THE GERMINATION OF SPORES DISSECTED IN ORDER FROM WHOLE ASCI SECURED FROM CROSSES OF LEUCINELESS-ADAPTED BY LEUCINELESS

CROSS:	$\alpha \times 33757-a$		$\beta \times 33757-a$				$\gamma \times 33757-4687-A^a$	
SPORE NO.	COLOR <sup>b</sup>	GROWTH <sup>c</sup> ON MINIMAL MEDIUM	COLOR	GROWTH ON MINIMAL MEDIUM	COLOR	GROWTH ON MINIMAL MEDIUM	COLOR	GROWTH ON MINIMAL MEDIUM
1	—	—	+	+	+	—	+	—
2	—	—	+	+	+	—	+	+
3	+	+	+	+	+	—	—	—
4	+	+	+	+	+	—	<sup>d</sup>	<sup>d</sup>
5	+	—	—	—	—	+	—	+
6	+	—	—	—	—	+	+	+
7	—	+	—	—	—	+	+	—
8	—	+	—	—	—	+	—	+

<sup>a</sup> This ascus was not dissected in order.

<sup>b</sup> + refers to salmon conidial pigment, — to albino.

<sup>c</sup> + refers to growth, — to failure to grow. All strains grew on medium to which leucine was added.

<sup>d</sup> This spore did not germinate.

In view of the 1:1 ratios obtained in these asci, leucine-independence in the adapted strains must be due to a chromosomal and not a non-Mendelian cytoplasmic factor. These crosses do not, however, supply any information about the relationship of the leucineless locus in strain 33757,  $l_1$ , to the gene for leucine-independence,  $L$ .

The demonstration of this relationship depends on crosses made between prototrophic strains from the  $f_1$  and wild type. In performing this cross, the  $f_1$  was used rather than the parent adapted strain to avoid the

confusion that might arise from the heterocaryotic<sup>6</sup> persistence of leucineless nuclei in the adapted culture. Barring mutation, a culture derived from a single ascospore is genetically homogeneous. The spores secured from these crosses are classified in table 2. The most extensive test was performed on the progeny of  $\beta$  where there were 13 asci in which every spore germinated, 5 asci in which at least one member of all four pairs of spores germinated so that each of the 4 chromatids could be accounted for, 66 spores from asci with incomplete germination and 83 spores isolated at random. In addition some data were secured on the progeny of crosses between wild-type and the prototrophic  $f_1$  strains secured from  $\alpha$  and  $\gamma$ . A total of 300 single-spore cultures was examined and every one proved to be prototrophic.

TABLE 2

CLASSIFICATION OF THE ORIGIN OF SINGLE-SPORE CULTURES FROM CROSSES OF PROTOTROPHIC  $f_1$  CULTURES WITH WILD TYPE. ALL CULTURES PROVED TO BE LEUCINE-INDEPENDENT

(SEE TABLE 1) $f_1$ FROM CROSS OF	×	WILD TYPE	NO. OF ASCI WHOSE 8 SPORES GERMINATED	NO. OF ASCI WITH ALL CHROMATIDS ACCOUNTED FOR (4-7 SPORES)	NO. OF SPORES FROM INCOMPLETE ASCI	NO. OF SPORES ISOLATED AT RANDOM
$\beta \times 33757-a$ (spore 1)		15300-A	0	3	10	0
$\beta \times 33757-a$ (spore 2)		15300-A	1	0	1	0
$\beta \times 33757-a$ (spore 3)		15300-A	12	2	47	83
$\beta \times 33757-a$ (spore 4)		15300-A	0	0	8	0
$\alpha \times 33757-a$ (spore 2)		15300-A	0	4	2	0
$\gamma \times 33757-4637-A$ (spore isolated at random)		43-14-A	0	3	7	0
			—	—	—	—
TOTAL			13	12	75	83

If leucine-independence in the adapted leucineless strains were due to mutation at a locus distinct from  $l_1$ , crosses between adapted and wild-type strains should have had in their progeny a recombination class of leucineless. In the 25 asci and among the 118 additional spores listed in table 2 there were no recombinations. Since these numbers test for 109 chances for recombination, of which none were fulfilled, the genes involved are probably alleles.

*Physiological Studies.*—The physiological behavior of the adapted strains confirms the hypothesis of reverse mutation. The rate of progression of the adapted strains and their prototrophic  $f_1$  progeny on an agar surface is the same as that of wild-type strains<sup>7</sup> from which they were originally derived (table 3). The addition of leucine did not stimulate or retard growth in either case. After 8½ days in 50 ml. of liquid medium containing 1 per cent sucrose, wild-type strain 1-A, albino strain 4637-A and adapted

strain  $\beta$  gave mycelial crops weighing between 109 and 114 mg. Thus, in their growth, as in their genetic behavior, leucineless-adapted cultures are indistinguishable from wild type. They probably represent back-mutations of the  $l_1$  locus to the wild-type allele.

TABLE 3

RATE OF GROWTH IN MM. PER HR. OF LEUCINELESS-ADAPTED COMPARED WITH THAT OF WILD TYPE AT 25°C. EACH RATE IS THE AVERAGE OF MEASUREMENTS ON TWO GROWTH TUBES

STRAIN	MINIMAL AGAR SUPPLEMENTED WITH		
	0	7.5 $\gamma$ l(+) LEUCINE PER ML.	2 MG. dl LEUCINE PER ML.
L-A	4.3	4.1	4.2
R977-a	4.0	...	...
4637-A	4.1	...	...
15300-A	4.4	4.2	...
$\alpha$	4.2	4.4	4.3
$\beta$	4.2	4.1	4.2
Leucine-independent spore 1 from $f_1$ of $\beta$ (see table 1)	4.2	4.2	4.3

*Are the Mutations Induced?*—In order to determine whether the back-mutations were induced an examination was made of the frequency of adaptation in liquid medium containing various amounts of leucine. Adaptations were identified by the arbitrary method previously described.<sup>3</sup> Those cultures which possessed unusually high weights, more than  $3\sigma$  above the mean of the other members of a series, were classified as adapted. The data are shown in table 4. The frequency of adaptations depended not only upon the temperature but was higher in low leucine concentrations than in high leucine concentrations. Both of these differences are significant by  $\chi^2$  test. Since the dependence upon leucine concentration appears to be an example of chemically induced mutation it is important to examine closely the events that take place during the  $8\frac{1}{2}$ -day period.

TABLE 4

EFFECT OF LEUCINE CONCENTRATION ON THE FREQUENCY OF ADAPTATIONS OF 33757-4637-A DURING  $8\frac{1}{2}$  DAYS IN 50-ML. LIQUID MEDIUM

TEMP: MG. l(+)LEUCINE	25°C.			30°C.			Av. % ADAPTATION
	No. of CULTURES	No. of ADAPTA- TIONS	% ADAPTATION	No. of CULTURES	No. of ADAPTA- TIONS	% ADAPTATION	
1.00	40	0	0	20	0	0	0
0.75	40	1	3	20	3	15	7
0.50	40	0	0	20	3	15	5
0.25	40	3	8	20	5	25	13
Av. % adaptation			3			14	

The leucine concentration expressed in table 4 is the initial concentration. As growth proceeds, the medium is depleted of leucine by the mold. When



the final weight is reached, bioassay of the medium proves the exhaustion of leucine. The medium will, at that time, support the growth of wild type *Neurospora*, or of leucineless *Neurospora* if further leucine is added. Thus, leucine alone is exhausted. The time at which this takes place depends upon the initial leucine concentration. On 0.25 mg. of *l* (+) leucine the final weight of about 7.5 mg. is attained in about 3 days. On 1.00 mg. *l* (+) leucine almost 6 days are required for the development of the final weight of 37 mg. During these times the mass of mycelium is increasing and with it, presumably, the number of nuclei in which back-mutation has a chance to occur.

In order to minimize the importance of the difference in time a long-term experiment was designed in which cultures of 33757-4637-*A* started their growth on 0.25 and 0.5 mg. *l* (+) leucine per 50 ml. at 30°C. The frequency of adaptation was observed between 6 and 45 days after inoculation, during which time all cultures were exposed to subthreshold leucine concentrations. Table 5 shows the results obtained. The same criterion was

TABLE 5

EFFECT OF LEUCINE CONCENTRATION ON THE FREQUENCY OF ADAPTATIONS OF 33757-4637-*A* BETWEEN 6 AND 45 DAYS IN 50-ML. LIQUID MEDIUM AT 30°C.

MG. <i>l</i> (+)LEUCINE	NO. OF CULTURES	NO. OF ADAPTATIONS	% ADAPTATION
0.50	43	5	12
0.25	38	16	42

used for identification of adaptations. It will be observed that even during this period there is a significantly higher frequency of adaptation in the cultures which began to grow in the presence of low leucine concentrations. Indeed, the frequency is so high that the average weight of all cultures, adapted and non-adapted, is higher (25 mg.) in the 0.25 mg. leucine series than in the 0.50 mg. leucine series (20 mg.). On the spontaneous mutation hypothesis one would expect the number of adaptations to be greater in those cultures with the higher nuclear populations, the high leucine series. We find the reverse to be true—the frequency of adaptations is an inverse function of mycelial mass.

The orthodox viewpoint is that adaptive mutations are not directed by the environment of the cell but occur in a given percentage of the population per unit time, and may be selected by the environment. We have shown that adaptation, in this instance, is a mutation phenomenon. Thus far we have assumed that when a mutation to leucine-independence takes place it will be regularly selected for in the absence of leucine and result in an adaptation. However, this is not the case. Although adaptations are the result of mutation, every mutation does not yield an adaptation as the evidence presented below will indicate.

*Heterocaryons.*—When a back-mutation occurs in a leucineless mycelium and the mutated nucleus multiplies a heterocaryon<sup>6</sup> is formed consisting of a mixture of leucineless and wild-type nuclei. The phenomenon of selection in such heterocaryons was studied by artificially preparing heterocaryons between leucineless and prototrophic strains and growing them on different concentrations of leucine. Figure 1 shows the behavior of a heterocaryon between the adapted strain  $\beta$ , and the leucineless strain, 33757-4637-A, which gave rise to it. Growth was measured on the surface of agar in growth tubes containing no leucine. The leucineless strain showed, of course, no growth. The prototrophic  $f_1$  control grew at the

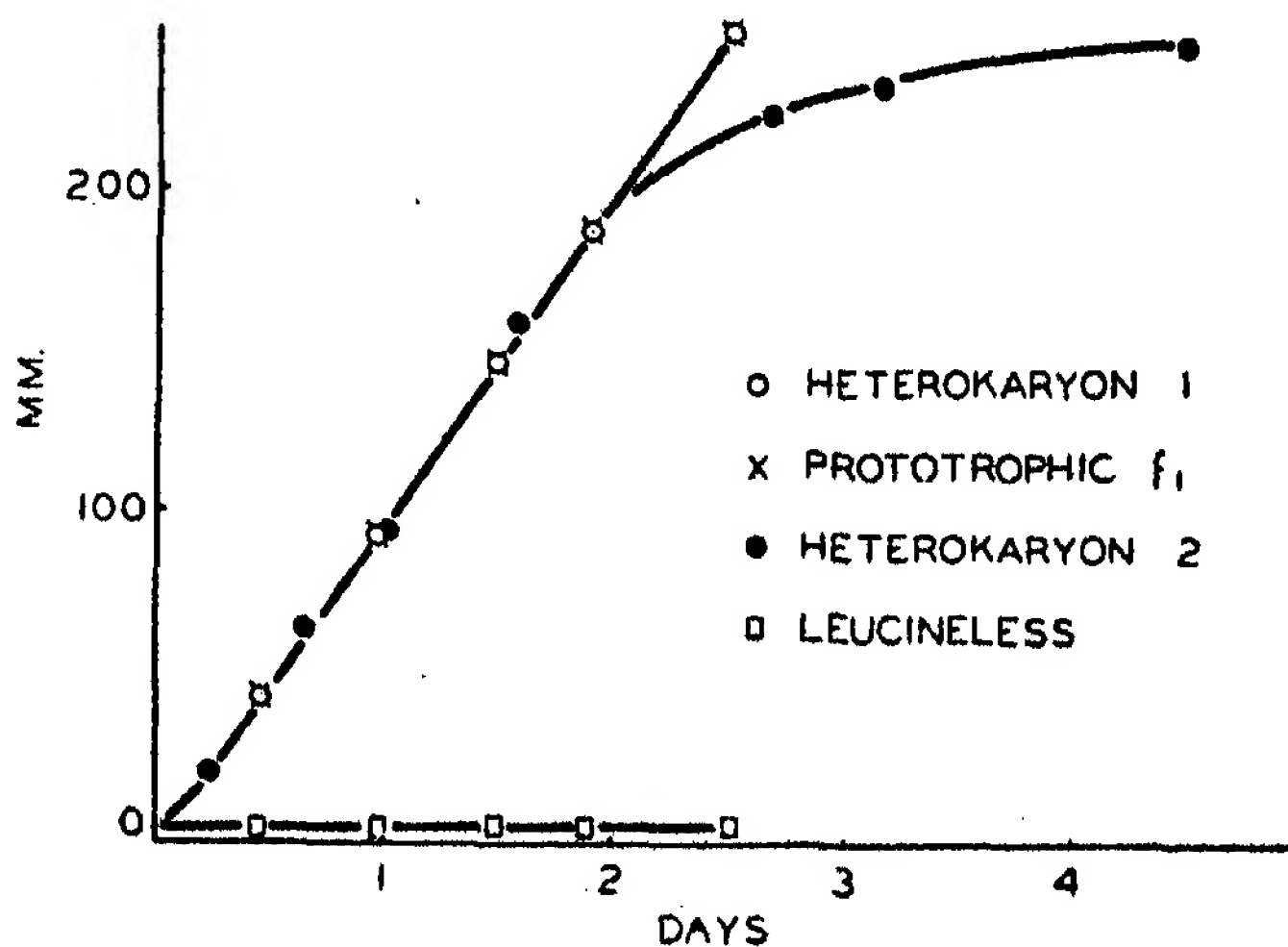


FIGURE 1  
Growth rate of a heterocaryon of leucineless and adapted *Neurospora* on minimal medium.

wild-type rate of 4.2 mm./hr. The heterocaryon grew at exactly the same rate, as though the wild-type nuclei it contained had outgrown the leucineless nuclei. On medium containing a limiting concentration of leucine the prototrophic  $f_1$  control still grew at a rate of 4.2 mm./hr. (Fig. 2). However, the heterocaryon grew at the same rate as the leucineless strain, 2.2 mm./hr. It appears as though the wild-type nuclei failed to outgrow the leucineless in the presence of a limiting leucine concentration. In other words, on minimal medium the heterocaryon grows like wild type but in the presence of leucine it grows like leucineless.

To prove that such behavior would be characteristic of hyphae known to be heterocaryotic, minimal agar plates were inoculated with a mixture of leucineless 33757-4637-A and of adapted strains  $\alpha$  or  $\beta$ . After the mycelium had grown over an area ca. 4 cm. in diameter, tips of single hyphae

were isolated<sup>6</sup> and transferred to media containing or lacking leucine. The plate from which these isolations were made was also kept for further observation. Hyphae isolated to minimal medium continued to grow (as did the parent mycelium on the agar plate) demonstrating that they contained nuclei of the adapted genotype. Since hyphal tips were transferred at random, such nuclei must also have been present in those hyphae inoculated into tubes of leucine-containing medium. The conidia formed in these tubes, however, did not grow when tested on minimal medium. Therefore, in the presence of leucine, hyphae which originally contained some leucine-independent nuclei gave rise to conidia which were purely

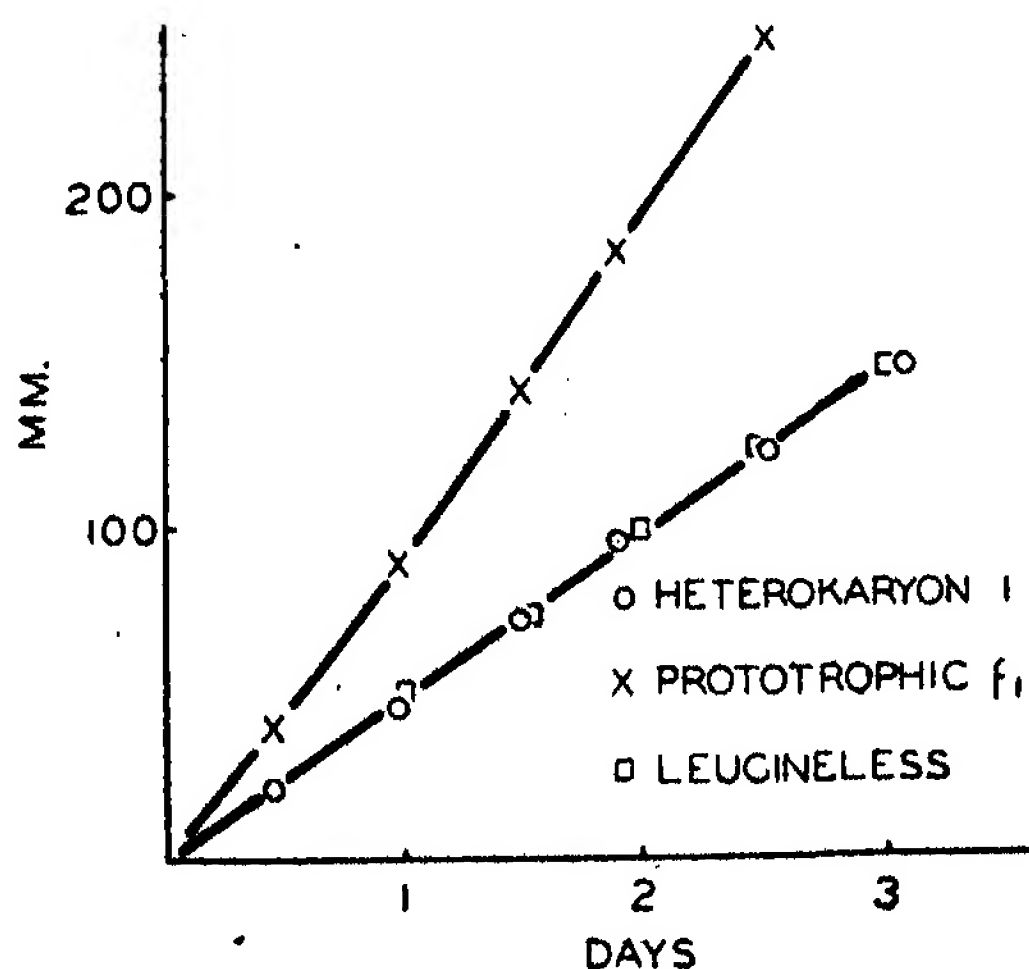


FIGURE 2  
Growth rate of a heterocaryon of leucineless and adapted *Neurospora* on a limiting concentration of  $l$  (+) leucine (0.0075 mg./ml.).

leucineless. Such behavior was characteristic of combinations of leucineless with wild-type strains obtained independently of the leucineless mutation. The similarity of adapted and wild-type strains in this respect is further evidence for the identity of the adapted and wild-type strains at the  $L$  locus.

This phenomenon is under further study but the following evidence may be reported briefly here. Hyphae were isolated in a similar manner from heterocaryons in which the leucineless and wild-type nuclei bore different marker-genes for conidial color. Such heterocaryotic hyphae behaved similarly to those described above. Furthermore, whenever a leucineless culture was selected from a heterocaryon, the color markers of the wild type could not be demonstrated. This is in favor of the hypothesis that

the wild-type nuclei in such combinations were selected against in the presence of leucine. The disappearance of color markers which characterized leucineless nuclei demonstrated a selection in favor of wild type when heterocaryons were grown on minimal medium. However, in some instances when heterocaryons were studied in growth tubes on minimal medium, they grew like wild type for a time but then slowed down and sometimes stopped (Fig. 1). This behavior helps to interpret the results of the long-term growth experiment in liquid medium.

In liquid cultures when a back-mutation to leucine independence occurs and a heterocaryon is formed the independent nuclei may overgrow and form a complete adaptation. On the other hand, the heterocaryon may begin to grow and then stop as the back-mutated nuclei are inactivated by the leucineless. This behavior was repeatedly observed during growth in liquid medium. In a culture which had reached the maximum growth for its leucine level a new patch of growing mycelium often appeared on the surface of the clot. This new growth may continue and overgrow the whole culture, or, after its initiation, it may stop, forming what was previously termed a "partial" adaptation.<sup>3</sup> Presumably we were observing heterocaryon selection.

The interpretation we offer for the higher frequency of adaptation in low leucine concentrations is in terms of the size of the mycelial mass involved rather than directly in terms of the leucine content of the medium. The greater the mycelial mass, the larger the population of leucineless nuclei in the midst of which a leucine-independent nucleus arises by mutation and the greater the chance that this mutant nucleus will be inactivated. Although we cannot duplicate directly the introduction of a single leucine-independent nucleus into a leucineless mycelium we have studied the growth of heterocaryons in liquid medium. Figure 3 shows the weights assumed after 8½ days by a heterocaryon of  $\beta$  and 33757-4637-A and by 33757-4637-A on different leucine concentrations.

As the leucine concentration rises the amount of mycelium, and hence the number of leucineless nuclei, similarly increases. However, the final weight of the heterocaryon decreases with leucine concentration. Apparently the greater the number of leucineless nuclei the more rapidly the leucine-independent nuclei are inactivated and the sooner growth ceases. These experiments, then, provide evidence for our interpretation of the frequency of adaptations on different leucine concentrations. An adaptation is due to the growth of leucine-independent nuclei which arose by mutation but whether the increased growth will be significant depends upon whether conditions favor the inactivation of the leucine-independent nuclei. Large mycelial masses with many leucineless nuclei are more unfavorable for escape of the prototrophic growth than small mycelial masses. We do not believe that our experiments allow any conclusion as to the rôle of

leucine in the induction of mutation at this locus. Such mutations probably occur spontaneously but their expression depends upon the leucine content of the environment through its effect on mycelial mass.

*Discussion.*—Because of the heterocaryon selection the back-mutation of locus  $l_1$  is not a favorable object for the study of mutation rates. For example, the effect of temperature shown in table 4 may be due to its influence either on the mutation rate or on the selection efficiency or both. Any calculation of mutation rate would yield a minimum value, at best, because many, if not most, mutations can never express themselves as adaptations because of the rapid inactivation of mutant leucine-independent nuclei.

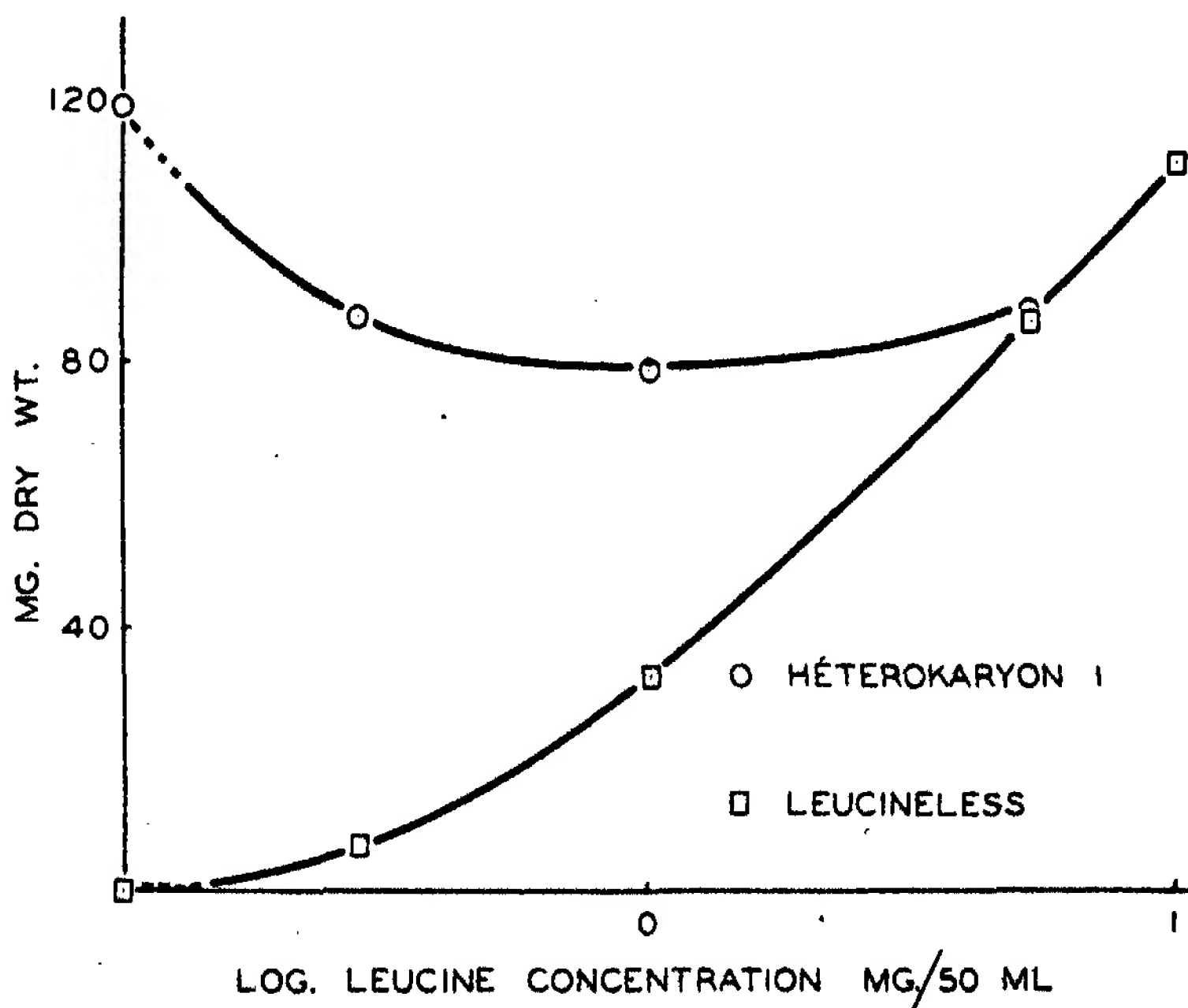


FIGURE 3

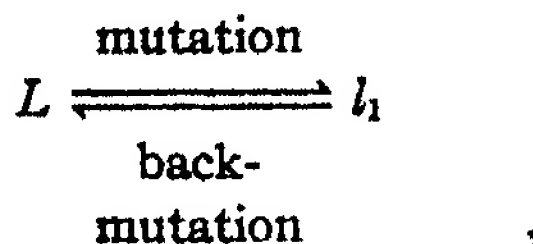
Final growth of a heterocaryon of leucineless and adapted *Neurospora* on different concentrations of  $l (+)$  leucine in liquid medium at 25° C.

On the basis of the selection phenomenon it is to be expected that back-mutations of locus  $l_1$  would not persist in stock cultures maintained on leucine. This probably explains the fact that in our experience no culture in stock tubes has ever adapted. Likewise in nature the heterocaryon selection phenomenon may help to maintain mutant types.

In bacteria nutritional mutants are known to adapt and lose their requirement.<sup>8</sup> In this way they seem to gain a new function. In the light



of recent evidence it is conceivable that nutritional mutants in bacteria are formed by an inactivation of a gene.<sup>9</sup> From this state it may later back-mutate. We conceive of this process in *Neurospora* as follows:



The wild-type allele,  $L$ , is present in the wild-type stock and controls some step in the synthesis of leucine. Under the influence of ultra-violet light, or otherwise,<sup>10</sup> this gene can mutate to the inactive state,  $l_1$ . We conceive that, where  $L$  makes an active enzyme involved in leucine synthesis and self-duplicates as well,  $l_1$  is also self-duplicating and could possibly make a "defective enzyme." The self-duplicating inactive gene  $l_1$  spontaneously back-mutates to  $L$ . We believe it to be established, with a fair degree of certainty, that the leucineless mutation in *Neurospora*,  $l_1$ , is not a chromosomal rearrangement or deficiency but a modification of a gene to a still self-duplicating particle.<sup>11</sup>

*Summary.*—The leucineless mutant of *Neurospora* is a true gene mutation. The adaptation to leucine-independence, which this mutant sometimes undergoes, is due to back-mutation to the wild-type condition at the leucineless locus. This is demonstrated by the genetic behavior of the adapted strain in crosses with leucineless and by the genetic behavior of the leucine-independent  $f_1$  progeny in crosses with wild type. Moreover, the adapted and wild-type strains are physiologically identical.

The incidence of adaptations is significantly higher in the presence of low concentrations of leucine than in the presence of high concentrations. This apparent chemical induction of mutations has its explanation in the fact that a back-mutation in the leucineless strain always results in the formation of a heterocaryon. In a heterocaryon between the leucineless and the adapted or wild-type strains, the leucineless nuclei have an advantage in the presence of leucine. Whether a back-mutation will result in an adaptation depends upon whether conditions favor selection against the leucine-independent nuclei.

<sup>1</sup> Beadle, G. W., *Physiol. Rev.*, 25, 643-663 (1945); Beadle, G. W., and Tatum, E. L., *Am. Jour. Bot.*, 32, 678-686 (1945).

<sup>2</sup> Regnery, D. C., *Jour. Biol. Chem.*, 154, 151-160 (1944).

<sup>3</sup> Ryan, F. J., and Brand, E., *Ibid.*, 154, 161-175 (1944).

<sup>4</sup> The experimental procedures used in these studies have been described previously. See references 1 and 3 and Ryan, F. J., Beadle, G. W., and Tatum, E. L., *Am. Jour. Bot.*, 30, 784-799 (1943).

<sup>5</sup> We propose to designate as a prototroph any strain which has the nutritional requirements of the "wild type" from which it was derived irrespective of how it became

prototrophic. (For *Neurospora crassa* see Butler, E. T., Robbins, W. J., and Dodge, B. O., *Science*, 94, 262 (1941).)

<sup>6</sup> Beadle, G. W., and Coonradt, V. L., *Genetics*, 29, 291-308 (1944).

<sup>7</sup> Different "wild-type" stocks undoubtedly carry gene differences which can modify such physiological characteristics as growth rate and degree of conidiation.<sup>4</sup> These differences may segregate to different stocks. Therefore, it is not strictly correct to speak of the wild type as an entirely distinctive genotype. However, in every mutant studied the nutritional requirement is determined by a single gene, although the genetic background may, to a certain extent, modify the details of its expression. It would be desirable to use "isogenic" strains, obtained by repeated back-crossing, but the biparental inheritance of *Neurospora* makes very difficult the elimination of any gene differences that may be linked to mating-type alleles.

<sup>8</sup> Roepke, R. R., Libby, R. L., and Small, M. H., *Jour. Bact.*, 48, 401-412 (1944).

<sup>9</sup> Gray, C. H., and Tatum, E. L., these PROCEEDINGS, 30, 404-410 (1944).

<sup>10</sup> The leucineless mutant, 33757, was obtained from a culture of *Neurospora* which had been treated with ultra-violet radiation. However, since parallel studies on spontaneous mutation were not carried out<sup>1</sup> it cannot be proved that the mutation  $L \rightarrow l_1$  was induced and did not occur spontaneously.

<sup>11</sup> Strain 4637-A is known to carry a translocation (McClintock, B., *Am. Jour. Bot.*, 32, 671-678 (1945)) which is closely linked to albino. It reduces crossing over in a region of the sex chromosome (Doermann, A. H., *Arch. Biochem.*, 5, 373-384 (1944)). The prototrophic  $f_1$  strain used in these experiments, although wild type in color, may have carried the translocation. The adaptation could have been due to mutation to leucine independence at a locus other than  $l_1$  since, in the cross of the  $f_1$  with wild type, reduction of crossing over might have prevented the appearance of recombinations. There are two types of evidence which militate against these assumptions. First, we found no reason to believe that the  $f_1$  by wild-type cross produced the lethal classes which would be expected if a translocation were involved. Second, the physiological identity of adapted strains with wild type speaks for the identity of the leucine-independent gene and the wild-type allele of  $l_1$ .

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## VARIETIES AND MATING TYPES IN *PARAMECIUM BURSARIA*. I. NEW VARIETY AND TYPES, FROM ENGLAND, IRELAND, AND CZECHOSLOVAKIA\*

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*Paramecium bursaria* consists of a number of varieties (Jennings,<sup>1</sup> Jennings and Opitz<sup>2</sup>). Members of any one variety do not, as a rule, mate with members of other varieties. In each variety there is a definite number of mating types. Animals of diverse mating types conjugate readily; animals of the same mating type usually do not mate together. Variety I contains four mating types designated A, B, C, and D. Variety II has eight mating types designated E, F, G, H, J, K, L, and M. Variety

III contains four mating types designated N, O, P, and Q. Variety IV, as far as it is known, contains two mating types designated R and S. In addition to these four varieties there are certain Russian clones (denominated Ru20 to Ru35) that do not conjugate with any of the four varieties (Jennings and Opitz<sup>2</sup>). These workers were of the opinion that these Russian clones possibly belong to a fifth variety. In the present paper they are called the fifth variety. (Apparently only one mating type of this variety has been found, and this type is designated T.) There are known, therefore, five varieties containing nineteen mating types in this species of *Paramecium*.

The present paper reports the finding of a new variety (Variety VI) containing four mating types. These animals were collected from England, Ireland, and Czechoslovakia.

*Material and Methods.*—In July and August, 1944, cultures of two clones of *P. bursaria* were received from Professor E. G. Pringsheim in Cambridge, England. One clone (Ck1) was collected in Prague, Czechoslovakia. It was first used in his extensive experimental studies while he was in Czechoslovakia; it was later brought by him to England. This clone has been in cultivation in the laboratory for approximately twenty years. The other clone (En1) was collected in 1944 in Cambridge, England.

In June, 1945, cultures of four additional English clones (En2, En3, En4, En5) were received from Professor Pringsheim. These were collected from Newnham, near Cambridge, England.

In June, 1945, a culture of *P. bursaria* from Dublin, Ireland, was also received from Professor Pringsheim. These animals were collected by Mr. Douglas Glen of Dublin and forwarded to the writer by Professor Pringsheim. A single animal was isolated from this culture and grown in isolation. This clone was designated as Ir1.

It is a pleasure to acknowledge my indebtedness to Mr. Glen and especially to Professor Pringsheim for sending these paramecia.

The animals were cultured in essentially the manner described by Jennings.<sup>1</sup> For cytological studies the animals were fixed in Schaudinn's fluid containing glacial acetic acid, stained in iron hematoxylin, and destained in saturated aqueous solution of picric acid, following the technique the writer has described previously (Chen<sup>3</sup>).

*Experimental Studies.*—1. *Determination of Variety and Mating Types:* Two series of tests were carried out on the Czechoslovakian, Irish, and English clones: (1) to determine whether these seven clones would conjugate with each other, and (2) to determine whether these clones belong to any of the five varieties already known.

To determine whether these seven clones would conjugate with each other, mixtures were made in all possible combinations of two of the seven clones. The results of these numerous tests are shown in table 1. An

analysis of the data shows that these seven clones belong to the same variety and that four mating types are present. Clones Ck1 and Ir1 belong to one mating type; they do not mate with each other but both mate with all other five clones. Clone En1 belongs to a second mating type; it mates with all other six clones. Clone En2 belongs to a third

TABLE 1

RESULTS OF MIXING IN ALL POSSIBLE COMBINATIONS OF TWO OF THE SEVEN CLONES OF *P. bursaria* COLLECTED FROM CZECHOSLOVAKIA, IRELAND, AND ENGLAND. THE PLUS SIGNS INDICATE THAT CLUMPING AND CONJUGATION OCCUR IN THE MIXTURE OF THE TWO CLONES INDICATED; THE MINUS SIGNS THAT THEY DO NOT

	Ck1	Ir 1	En 1	En2	En3	En4	En5
Ck1	—	—	+	+	+	+	+
Ir1	—	—	+	+	+	+	+
En1	+	+	—	+	+	+	+
En2	+	+	+	—	+	+	+
En3	+	+	+	+	—	—	—
En4	+	+	+	+	—	—	—
En5	+	+	+	+	—	—	—

mating type; it also mates with all other six clones. Clones En3, En4, and En5 belong to a fourth mating type; they do not mate with each other but they all mate with the other four clones.

Under proper conditions, the animals of diverse mating types when mixed exhibited the agglutinative mating reaction immediately. In a few

minutes large clots or masses of animals were formed. Later these clots and masses broke up and many conjugating pairs were found. If a large number of animals of these clones were mixed, hundreds of pairs could be found in a single mixture. The behavior in mating reaction and pair formation as well as the nuclear changes during conjugation (described later in this paper) appeared to be normal and typical of this species.

Many tests were carried out to determine whether the seven clones would mate with animals belonging to any of the five already known varieties. These tests were carried out in the following manner. Cultures of many clones belonging to all of the nineteen known mating types constituting the five varieties were kept in the laboratory. These are known as "testers"; while the cultures of the Czechoslovakian, Irish, and English clones were designated "unknown." Before testing, a number of animals were taken out from one "tester" and mixed with those of another "tester" of an appropriate mating type (animals of a diverse mating type belonging to the same variety) to determine whether the "testers" were in a sexually reactive condition. The animals were considered sexually reactive if when mixed they would agglutinate immediately and form pairs. Animals of the "unknown" clones were similarly tested with each other. Tests between the "unknown" and the "testers" were carried out only when both were sexually reactive. In actual testing a number of animals of an unknown clone were mixed separately with animals from different mating type "testers." These mixtures were examined a few hours after preparation and also examined daily during the following four days to determine whether any pairs or clots were formed.

Representative clones of two of the four mating types (the Czechoslovakian clone Ck1 and the English clone En1) were tested separately with all four mating types of Variety I, seven of the eight mating types of Variety II, two of the four mating types of Variety III, the two mating types of Variety IV and the single mating type of Variety V. Altogether 77 tests were carried out on the Czechoslovakian clone, and an equal number on the English clone En1.

The results of these tests can be summarized as follows: No mating reaction or conjugation was ever found in any of the tests except in some of the cases when the English clone and a fifth variety clone (Ru22) were mixed. In such a mixture pairs could be found but most of these pairs were atypical in that the two conjugants did not have the relative positions typical of normal conjugating pairs. It was soon found out that there was really no conjugation between the English clone and the fifth variety; the conjugating pairs each consisted of two English animals, the fifth variety animals taking no part in the conjugation. (The pair formation was induced by the fluid of the fifth variety.<sup>4</sup>) This non-conjugation between English animals and the fifth variety was proved in the following manner:



(1) When dark green English animals were mixed with white fifth variety animals, every pair consisted of two dark green animals. (2) The individuals of the English clone are much smaller than the fifth variety animals. When the English and the fifth variety animals were mixed, each pair consisted of two small animals of similar size. (3) There are differences in the shape of the micronuclei between the English clone and the fifth variety, the latter having a much longer micronucleus. Fixed and stained pairs from a mixture of English and the fifth variety animals show that the micronuclei in two conjugants of each pair were alike; both micronuclei were short. (4) As already stated, although some of the pairs found in a mixture of English and the fifth variety animals are typical, others are atypical in that the two conjugants do not have the relative positions typical of normal conjugating pairs. If the English animals were placed in the fluid of the fifth variety, similar pairs (mostly atypical) were formed.<sup>4</sup>

Judging from the results of the numerous tests just described, it is obvious that these two clones do not belong to any of the five known varieties. They, together with the other five clones, clearly constitute a new variety containing at least four mating types. This new variety is to be known as the sixth variety, and the four new mating types are designated as types U, V, W, and X. Thus, there are now known in this species of *Paramecium* six varieties containing twenty-three mating types, which are diagrammatically presented in figure 1. The twenty-three types are designated by capital letters A-X. The small letters or figures in the lower half of the squares are designations of the clones exemplifying each mating type.

2. *Temporary Pair Formation:* With the exception of the Irish clone (Ir1) all clones of this variety are normal in that when they are mixed with clones of a different mating type, lasting pairs are formed. When the Irish clone is mixed with normal clones of a different mating type the pairs that are formed are not lasting but separate within a few hours. For example, if the Irish clone is mixed with clone En2 at about noon, strong agglutinative mating reaction occurs almost immediately. Clots of animals are formed within a few minutes. Within an hour or two, many pairs are formed but these pairs are not lasting. During the late afternoon and in the evening the pairs break up into single animals. If such a mixture is placed in a moist chamber and kept from drying, the typical agglutinative mating reaction and temporary pair formation recur the following day and daily for some successive days (perhaps for many days). The following is the characteristic daily behavior of the animals in such a mixture. The agglutinative mating reaction begins at about noon. Many pairs are formed in the early afternoon. In the late afternoon the pairs begin to break up into single animals so that by evening only a few pairs may be found; none are present by eleven o'clock P. M.

Similar phenomena are observed if the Irish clone is mixed with the following clones of the new variety: En1, En3, En4, and En5.

The temporary pair formation described in the present paper is similar to that reported by Sonneborn<sup>6</sup> in *P. aurelia* and by Jennings<sup>6</sup> and Chen<sup>7</sup> in *P. bursaria*.

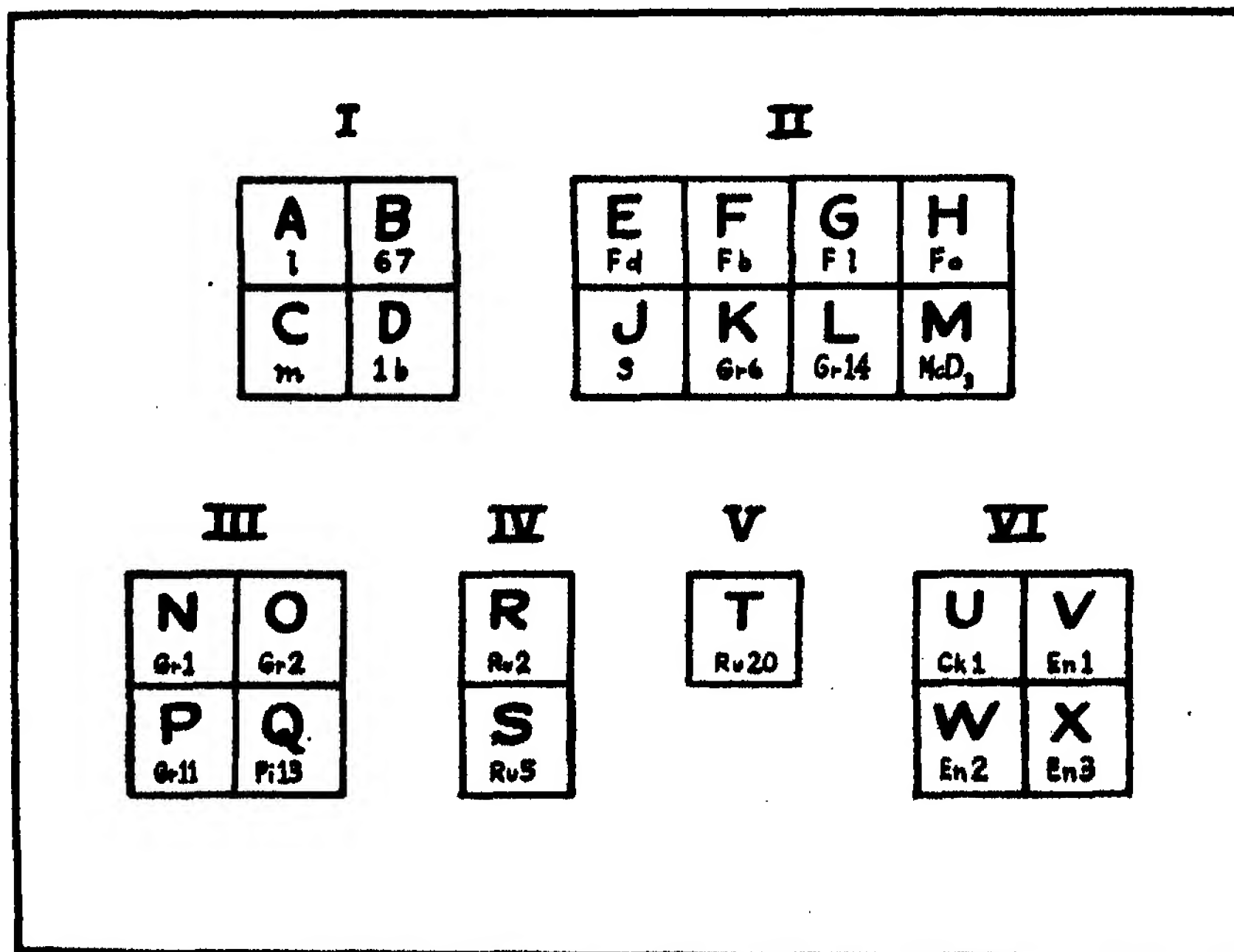


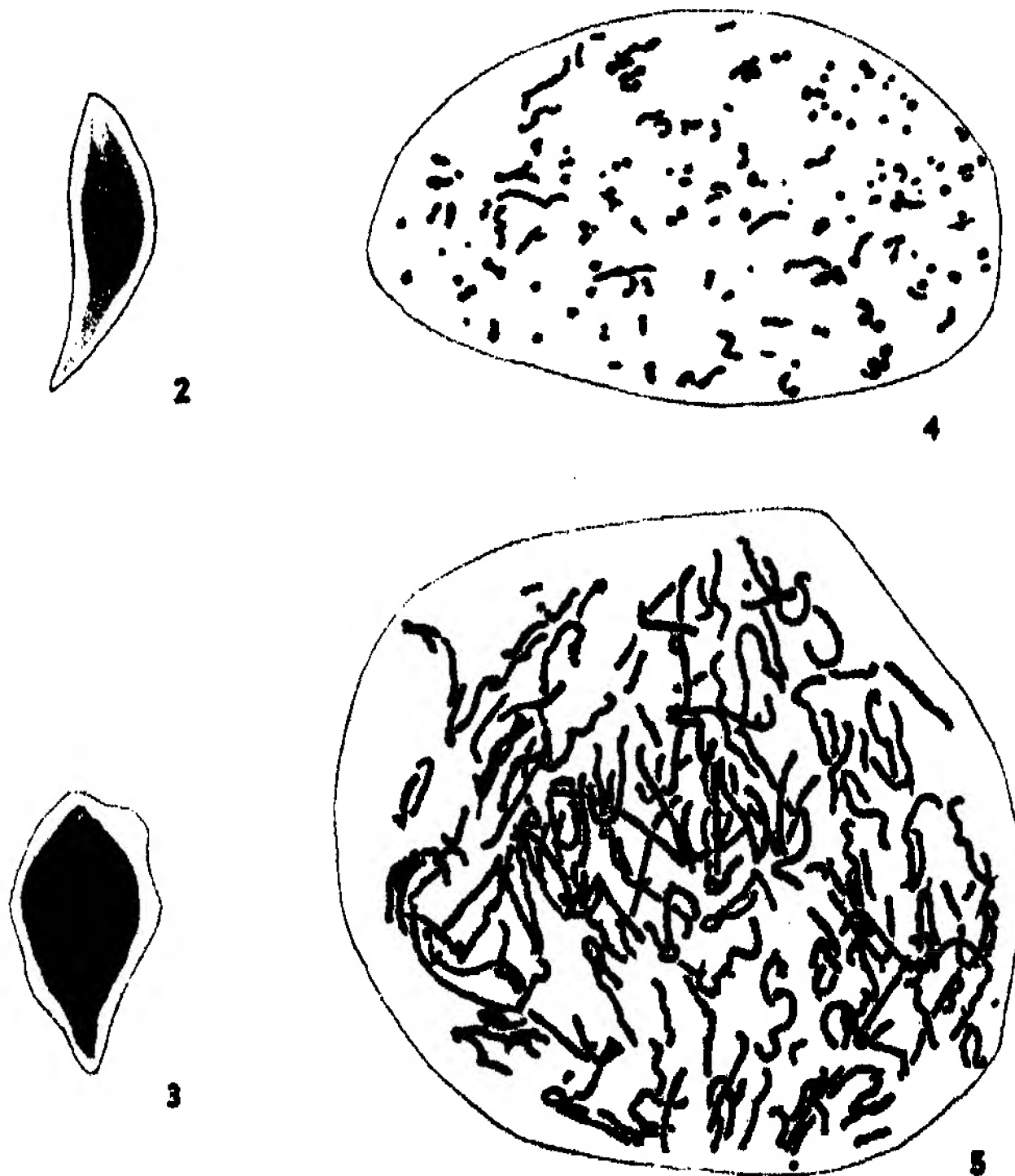
FIGURE 1

Diagram of the six known non-interbreeding varieties of *P. bursaria*. The capital letters are the designations of mating types in each variety: four in Variety I, eight in Variety II, four in Variety III, two in Variety IV, four in Variety VI. In Variety V apparently only one mating type is known. The figures or numbers in the lower half of the squares are the designations of clones that first exemplified each mating type.

**Cytological Studies.**—Cytological studies were carried out to determine whether nuclear changes during conjugation in this variety are normal. The Czechoslovakian clone Ck1 (mating type U) and the English clone En1 (type V) were used.

These two clones differ greatly in their micronuclei and in their chromosomes. The Czechoslovakian clone has a very small, lightly staining, ellipsoidal micronucleus (Fig. 2), whereas the English clone has a much larger and deeply staining micronucleus containing a much greater quantity

of chromatin (Fig. 3). As is seen clearly during the late prophase of the first pregamic division in conjugation, the chromosomes of the Czechoslovakian clone are for the most part either spherical or very short rods (Fig. 4), whereas most of the chromosomes of the English clone are much longer (Fig. 5). In this connection, it may be stated that this is the first variety in which I have found such great chromosomal differences between



FIGURES 2 TO 5

Figure 2, resting micronucleus of a vegetative animal belonging to clone Ck1. Figure 3, resting micronucleus of a vegetative animal belonging to clone En1. Figure 4, chromosomes of clone Ck1 as seen during the late prophase of the first pregamic division in conjugation. Figure 5, chromosomes of clone En1 as seen during the late prophase of the first pregamic division in conjugation. All figures  $\times 1640$ .

clones of the same variety. In my experience, the chromosomes of clones of the same variety are similar, though marked chromosomal differences may exist between varieties. For example, Varieties II and IV are characterized by the presence of thin and short chromosomes, whereas the chromosomes in Variety III are larger and much longer.

1. *Nuclear Changes during Conjugation:* Nuclear changes during con-

jugation between these two clones are normal and typical of the species. They include three pregamic divisions, the exchange and fusion of pronuclei, and the three post-zygotic divisions. In the following paragraphs these nuclear changes and the time relationships of stages (at 26°C.) will be described.

The first pregamic division is a long process requiring approximately 24 hours for completion. The daughter nuclei formed as a result of the first pregamic division are similar in size and structure. Soon one of the nuclei degenerates, and the other remains to undergo the second pregamic division.

The second pregamic division occurs 25–27 hours after the onset of conjugation and is consummated in a short time—perhaps in one or two hours. The nuclei formed as a result of the second pregamic division are also similar in size and structure. Soon one of the nuclei degenerates; the remaining nucleus undergoes the third pregamic division.

The third pregamic division generally occurs at 28–30 hours. Of the two pronuclei formed as a result of the third pregamic division, one is migratory, the other stationary. Most cases of exchange of pronuclei were found at 30–32 hours, although some may be found as early as 29 hours.

After exchange, the two pronuclei in each conjugant fuse and form a synkaryon. Three post-zygotic divisions take place. The first division occurs at 32–33 hours. One of the two nuclei resulting from this division degenerates, and two further nuclear divisions occur at 34–38 hours. By the 38th hour, ex-conjugants are found. Later two of the nuclei in the ex-conjugants develop into macronuclear anlagen, the other two become the micronuclei.

Even though the nuclear changes during conjugation between the English and Czechoslovakian clones are typical of this species of *Paramecium*, there occur conjugants and ex-conjugants in which the nuclear conditions are abnormal, and the number of the abnormal animals is unusually high. Some of the abnormal nuclear conditions can be listed: (1) There are a number of conjugants (mostly Czechoslovakian conjugants) in which both of the nuclei resulting from the first pregamic division persist and undergo the second pregamic division. (2) There are many conjugants in which the pronuclei, after exchange, do not fuse together to form a synkaryon but each pronucleus develops into a hemikaryon.<sup>6</sup> (3) The number of nuclei in the ex-conjugants is unusually variable. In this species of *Paramecium* the normal number of nuclei in the ex-conjugants immediately after separation is four (two of these later develop into macronuclear anlagen, the other two become the micronuclei). Among the ex-conjugants resulting from conjugation between the English and Czechoslovakian clones approximately fifty per cent of the animals have the normal number of nuclei;

in the other fifty per cent the number of nuclei varies from two to twenty. It is difficult to explain the unusually high percentage of abnormal conjugants and ex-conjugants. Possibly it is due to some degree of incompatibility between these two clones or to the fact that one of these two clones (Ck1) is a very old clone, having been cultured in the laboratory for approximately twenty years. The English clone at the time of mating with the Czechoslovakian clone was about a year old. Jennings<sup>9</sup> found that in *P. bursaria* when an old clone conjugates with a young one, most or all of the ex-conjugants die (just as when two old clones conjugate together).

2. *Differences between Varieties in the Rate of Nuclear Changes during Conjugation:* In *P. bursaria* there appear to be marked differences between certain varieties in the rate of nuclear changes during conjugation. For example, under comparable conditions the rate of nuclear changes during conjugation in Variety I is much faster than that in Variety II (at least in the cases studied). At 26.5°C. all of the nuclear changes during conjugation in Variety I are completed in 20–22 hours, whereas those in Variety II continue for 36 or more hours. In Variety I the first pre-gamic division requires but 12 or 13 hours for completion, whereas in Variety II it requires 20 or 21 hours.

The rate of nuclear changes during conjugation in the new variety (VI) is comparable to that of Variety II; it is certainly much slower than that of Variety I.

\* This work has been aided by grants from the Committee for Research in Problems of Sex, National Research Council; from the Joseph Henry Fund of the National Academy of Sciences; and from the Permanent Science Fund of the American Academy of Arts and Sciences.

<sup>1</sup> Jennings, H. S., *Genetics*, 24, 202–233 (1939).

<sup>2</sup> Jennings, H. S., and Opitz, P., *Ibid.*, 29, 576–583 (1944).

<sup>3</sup> Chen, T. T., *Stain Techn.*, 19, 83–90 (1944).

<sup>4</sup> Chen, T. T., *Proc. Nat. Acad. Sci.*, 31, 404–410 (1945).

<sup>5</sup> Sonneborn, T. M., *Anat. Rec.*, 84, 542–543 (1942).

<sup>6</sup> Jennings, H. S., *Biol. Bull.*, 86, 131–145 (1944).

<sup>7</sup> Chen, T. T., *Ibid.* (in press).

<sup>8</sup> Chen, T. T., *Jour. Hered.*, 31, 185–196 (1940).

<sup>9</sup> Jennings, H. S., *Jour. Exp. Zool.*, 96, 243–273 (1944).



## ON BLOCKS OF CHARACTERS OF GROUPS OF FINITE ORDER, I

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1. The present paper is a continuation of an earlier investigation.<sup>1</sup> Let  $G$  be a group of finite order  $g$ , and let  $\Gamma = \Gamma(G)$  denote the corresponding group ring formed with regard to an algebraic number field  $K$ . We shall assume that all the simple constituents of the semisimple ring  $\Gamma$  split completely in  $K$ . This hypothesis holds, for example, when  $K$  contains the  $g$ th roots of unity.<sup>2</sup> Let  $p$  be a rational prime number and let  $\mathfrak{p}$  be a prime ideal of  $K$  dividing  $p$ . The ordinary irreducible characters  $\zeta_\mu$  of  $G$  and the modular characters  $\varphi_\nu$  of  $G$  (for  $p$ ) are distributed into a certain number of "blocks"<sup>3</sup>  $B_1, B_2, \dots, B_n$ , each  $\zeta_\mu$  and each  $\varphi_\nu$  belonging to exactly one block  $B_r$ . As was mentioned in A G R, these blocks are linked closely with the arithmetic in  $\Gamma$ .

We are interested in obtaining relations between the blocks of  $G$  and those of certain subgroups  $N$ . These  $N$  will be the normalizers of the  $p$ -subgroups of  $G$  (and some related groups). This will mean that a number of important features of the characters of  $G$  are determined by the structure of these groups  $N$  and the position of  $N$  in  $G$ , in particular, the manner in which the classes of conjugate elements of  $N$  are distributed in the classes of  $G$ .

2. Denote the center of the group ring  $\Gamma = \Gamma(G)$  by  $\Lambda = \Lambda(G)$ . As is well known, a basis of  $\Lambda$  is formed by the classes of conjugate elements  $K_1, K_2, \dots, K_k$  of  $G$ , each class  $K_i$  being interpreted as the sum of all its elements. We then have formulae

$$K_\alpha K_\beta = \sum a_{\alpha\beta\gamma} K_\gamma \quad (1)$$

where the  $a_{\alpha\beta\gamma}$  are rational integers,  $a_{\alpha\beta\gamma} \geq 0$ .

Let  $H$  be any subgroup of  $G$  of an order  $p^h$ ,  $h \geq 0$ , where  $p$  is the fixed prime selected above. Denote by  $\mathfrak{C}(H)$  the centralizer of  $H$  in  $G$  and by  $\mathfrak{N}(H)$  the normalizer of  $H$  in  $G$ , and consider a subgroup  $N$  which satisfies the condition

$$H\mathfrak{C}(H) \subseteq N \subseteq \mathfrak{N}(H). \quad (2)$$

If  $K_\alpha^0$  is the part of  $K_\alpha$  which lies in  $\mathfrak{C}(H)$ , then either  $K_\alpha^0 = 0$  if  $K$  does not contain any elements of  $\mathfrak{C}(H)$ , or  $K_\alpha^0$  is a sum of complete classes of  $N$ . It can be shown easily that (1) implies

$$K_\alpha^0 K_\beta^0 \equiv \sum_\gamma a_{\alpha\beta\gamma} K_\gamma^0 \pmod{p}. \quad (3)$$

Consequently, the classes  $K_\alpha$  with  $K_\alpha^0 = 0$  form the basis of an ideal  $T^*$

of the center  $\Lambda^*$  of the modular group ring  $\Gamma^*$ .<sup>4</sup> On the other hand, the  $K_\alpha^0 \neq 0$  can be considered as the basis of a subring  $R^*$  of the center  $\Lambda^*(N)$  of the modular group ring  $\Gamma^*(N)$  of  $N$ . Now (3) yields

$$R^* \simeq \Lambda^*(G)/T^*. \quad (4)$$

This relation represents a connection between the group rings of  $G$  and of  $N$ ; it forms the basis of our work.

3. The algebra  $\Lambda^*(N)$  is commutative and splits completely, its irreducible characters  $\bar{\omega}^*$  are all linear. The character  $\bar{\omega}^*$  of  $\Lambda^*(N)$  induces a character of the subring  $R^*$ . Because of (4), this character may be interpreted as a character of  $\Lambda^*(G)/T^*$  and hence it induces a character  $\omega^*$  of  $\Lambda^*(G)$  which vanishes for the elements of  $T^*$ . If we know how the classes of  $N$  are distributed among the classes of  $G$ , we can express  $\omega^*$  explicitly in terms of  $\bar{\omega}^*$ . We have

$$\omega^*(K_\alpha) \equiv \sum \bar{\omega}^*(\tilde{K}_\rho) \pmod{\mathfrak{p}} \quad (5)$$

where  $\tilde{K}_\rho$  ranges over all classes of  $N$  which belong to  $K_\alpha$ .

Every ordinary character  $\zeta_\mu$  of  $G$  determines a character  $\omega_\mu$  of  $\Lambda(G)$  which is given by

$$\omega_\mu(K_\alpha) = g\zeta_\mu(\sigma_\alpha)/n_\alpha z_\mu \quad (6)$$

where  $\sigma_\alpha$  is an element in the class  $K_\alpha$ ,  $n_\alpha$  is the order of the normalizer of  $\sigma_\alpha$ , and  $z_\mu$  is the degree of  $\zeta_\mu$ . The modular characters  $\omega^*$  of  $\Lambda^*(G)$  are obtained by considering the different  $\omega_\mu \pmod{\mathfrak{p}}$ . In particular, two characters  $\zeta_\mu$  and  $\zeta_\nu$  belong to the same block  $B_r$ , if they yield the same  $\omega^*$ .

If  $\tilde{B}_r$  is a block of characters of  $N$ , there is associated a modular character  $\bar{\omega}^*$  of  $\Lambda^*(N)$  with  $\tilde{B}_r$ . As described above, this character  $\bar{\omega}^*$  determines a character  $\omega^*$  of  $\Lambda(G)$ . Again, this character  $\omega^*$  determines a block  $B_r$  of  $G$ . We shall say that  $B_r$  is the block of  $G$  determined by the block  $\tilde{B}_r$  of  $N$ . It follows from the results of A G R that the defect  $\tilde{d}_r$  of  $\tilde{B}_r$  and the defect  $d_r$  of  $B_r$  satisfy the inequality

$$h \leq \tilde{d}_r \leq d_r \quad (7)$$

where  $p^h$  is the order of  $H$ .

4. In (2), the group  $N$  was left arbitrary to some extent. Choose  $N$  now as the normalizer  $\mathfrak{N}(H)$  of  $H$ . It was shown in A G R that for a given block  $B_r$  of  $G$ , there exist subgroups  $H$  and blocks  $\tilde{B}_r$  of  $\mathfrak{N}(H)$  for which the equality sign holds in (7). If we consider conjugate subgroups of  $G$  as not essentially different, then  $H$  is uniquely determined. We call this group  $H$  the defect group  $H_r$  of  $B_r$ ; its order is  $p^h$  with  $h = d_r$ . Again, the block  $\tilde{B}_r$  of  $\mathfrak{N}(H_r)$  is uniquely determined.

Returning to the case of an arbitrary  $N$  in (2), we state

**THEOREM 1:** *Let  $H$  be a subgroup of order  $p^h$  of  $G$ , let  $N$  be a subgroup of  $G$  satisfying  $H \cdot \mathfrak{C}(H) \subseteq N \subseteq \mathfrak{N}(H)$ . If the block  $\tilde{B}_e$  of  $N$  with the defect group  $\tilde{H}_e$  determines the block  $B_r$  of  $G$  with the defect group  $H_r$ , then  $H \subseteq \tilde{H}_e \subseteq N$ , and  $\tilde{H}_e$  is conjugate in  $G$  to a subgroup of  $H_r$ .*

5. The  $k$  linear characters  $\omega_i$  corresponding to the  $k$  irreducible characters  $\zeta_i$  of  $G$  can be arranged in form of a matrix  $\Omega = (\omega_i(K_j))$  of degree  $k$ . If the block  $B_r$  contains  $x_r$  ordinary characters  $\zeta_i$ , then  $x_r$  rows of  $\Omega$  correspond to  $B_r$ . Choose a minor  $\Delta_r$  of degree  $x_r$  containing these  $x_r$  rows such that  $\Delta_r$  is divisible by  $p$  to the least possible power. It can then be shown that it is possible to make this selection of  $x_r$  columns for each block  $B_r$  in such a manner that every column appears for one and only one block. This result is by no means trivial; for the proof, the theory of algebras and the significance of blocks must be used.

Since the columns of  $\Omega$  correspond to the classes  $K_j$  of  $G$ , we have associated  $x_r$  classes  $K_j$  with every block  $B_r$  such that every class is associated with one and only one block. The selection of classes for the different blocks may be possible in more than one way. In any case, the number of  $p$ -regular classes among the classes associated with  $B_r$  can be shown to be equal to the number  $y_r$  of modular characters in  $B_r$ , and further these  $y_r$   $p$ -regular classes associated with  $B_r$  form a selection in the sense of A G R, § 3, in particular Theorem 2.

So far we assumed that  $B_1, B_2, \dots, B_t$  were the blocks of ordinary and modular characters of  $G$ . It will be important to note that the results of this section remain valid if every  $B_r$  is a collection of ordinary and modular characters of  $G$ , such that every ordinary and modular character of  $G$  belongs to exactly one  $B_r$ , and that every  $B_r$  consists of one or several blocks of  $G$ . Again  $x_r$  denotes the number of ordinary characters and  $y_r$  the number of modular characters in  $B_r$ .

6. We shall say that a group  $H$  of order  $p^h$  is the *defect group* of a class  $K_j$ , if  $H$  is a  $p$ -Sylow-subgroup of the normalizer of suitable elements of  $K_j$ . This implies that  $p^h$  is the highest power of  $p$  dividing  $n_j$  in (6); the exponent  $h$  will be termed the *defect* of  $K_j$ . We can now state the following results

**THEOREM 2:** *Let  $(\mathfrak{S})$  be a system of subgroups  $H$  of orders  $1, p, p^2, \dots$ , of  $G$  such that every subgroup of order  $p^h$  of  $G$  is conjugate to exactly one  $H$  in  $(\mathfrak{S})$ . For every  $H$  in  $(\mathfrak{S})$ , find the collection  $\tilde{B}^{(r)}$  of all blocks  $\tilde{B}_e$  of  $\mathfrak{N}(H)$  which determine a given block  $B_r$  of  $G$ , and select a full system of classes  $\tilde{K}_e$  of  $\mathfrak{N}(H)$ , which are associated with  $\tilde{B}^{(r)}$ . Suppose that  $r_e(H)$  of these classes  $\tilde{K}_e$  have  $H$  as their defect group. Different ones of these  $r_e(H)$  classes  $\tilde{K}_e$  belong to different classes  $K_a$  of  $G$ ; the classes  $K_a$  thus obtained for the different  $H$  in  $(\mathfrak{S})$  form a possible selection of classes associated with  $B_r$ .*

As corollaries, we have

THEOREM 3: The number of characters in  $B_r$  is given by

$$x_r = \sum_H r_r(H) \quad (8)$$

where the sum extends over all  $H$  in  $(\mathfrak{S})$ .

THEOREM 4: If  $s_r(H)$  of the  $r_r(H)$  classes  $\tilde{K}_p$  in Theorem 2 are  $p$ -regular, then the number of modular characters in  $B_r$  is given by

$$y_r = \sum_H s_r(H) \quad (9)$$

where  $H$  again ranges over all groups of  $(\mathfrak{S})$ .

THEOREM 5: If, in (9),  $H$  ranges only over those groups of  $(\mathfrak{S})$  which have a fixed order  $p^h$ , the corresponding sum

$$y_r^{(h)} = \sum s_r(H), (H \text{ in } (\mathfrak{S}); (H:1) = p^h) \quad (10)$$

represents the multiplicity of  $p^h$  as an elementary divisor of the Cartan matrix  $C_r$  of the block  $B_r$ .

It would be conceivable that the numbers  $r_r(H)$  and  $s_r(H)$  depend on the special selection of classes of  $\mathfrak{N}(H)$  associated with  $B_r$ . However, this is not so; we have

THEOREM 6: The numbers  $r_r(H)$  and  $s_r(H)$  in the preceding theorems depend only on the group  $G$ , the subgroup  $H$  and the block  $B_r$  of  $G$ .

7. In order to discuss our results, let us assume for the sake of simplicity that  $G$  does not contain any normal subgroup of an order  $p^h > 1$ . Suppose we know: (a) A complete system of subgroups  $H$  of a  $p$ -Sylow-subgroup of  $G$ , (b) which of the groups  $H$  in (a) are conjugate in  $G$ ; (c) the characters of the normalizers  $\mathfrak{N}(H)$ ,  $H \neq 1$ , (d) the manner in which the classes of conjugate elements of  $\mathfrak{N}(H)$  appear in the classes of conjugate elements of  $G$ .

If  $H \neq 1$ , then, under our present assumption,  $\mathfrak{N}(H)$  is a proper subgroup of  $G$ . If we know the characters, we can find the modular characters  $\tilde{\omega}^*$  of the center  $\Lambda^*(\mathfrak{N}(H))$  of the group ring of  $\mathfrak{N}(H)$ , and this gives us the blocks  $\tilde{B}_r$  of  $\mathfrak{N}(H)$ . Then (5) gives the modular characters  $\omega^*$  of  $\Lambda^*(G)$ . In this manner, all the characters  $\omega^*$  belonging to the different blocks  $B_r$  of positive defect are obtained. Further, we can determine which  $\tilde{B}_r$  for a fixed  $H \neq 1$  belong to  $\tilde{B}^{(\tau)}$ , and then find  $r_r(H)$  and  $s_r(H)$ . This is not sufficient to determine  $x_r$  and  $y_r$  completely, since the numbers  $r_r(1)$  and  $s_r(1)$  remain undetermined. However, we obtain lower bounds for  $x_r$  and  $y_r$ . Further, since any  $p$ -singular class  $K_\alpha$  has a positive defect, we have  $r_r(1) = s_r(1)$ , and hence the excess  $x_r - y_r$  of the number  $x_r$  of ordinary characters over the number  $y_r$  of modular characters in  $B_r$  can be obtained. Finally (10) gives the multiplicity of the elementary divisors different from 1 of  $C_r$ . This shows that a number of the most important

invariants of the blocks are determined by the information contained in (a), (b), (c), (d).

8. It had been shown in A G R, that if for a subgroup  $H$  of order  $p^h$  in  $G$ , the group  $\mathfrak{N}(H)$  contains  $q(H)$  blocks of defect  $h$ , then  $G$  possesses

$$\sum_H q(H), (H \text{ in } (\mathfrak{R}), (H:1) = p^h) \quad (11)$$

blocks of defect  $h$ . It may be remarked that the number  $q(H)$  can be determined by means of the group  $\mathfrak{N}(H)/N = U$  and its normal subgroup  $H\mathfrak{C}(H)/H = V$ . The characters  $\theta$  of  $V$  are distributed in classes of characters which are associated with regard to  $U$ ; two characters  $\theta$  and  $\theta_1$  being associated if

$$\theta_1(\sigma) = \theta(u^{-1}\sigma u)$$

where  $\sigma$  is a variable element of  $V$  and  $u$  is a fixed element of  $U$ . Then it can be shown that  $q(H)$  is equal to the number of classes of associated characters  $\theta$  of  $V$  of defect 0, such that no element  $u$  of  $U$  exists of order  $p$  with regard to the subgroup  $V$  for which  $\theta(u^{-1}\sigma u) = \theta(\sigma)$ . If  $h > 0$ , this result requires only the investigation of groups of smaller order than  $g$ , in order to obtain  $q(H)$  and (11).

9. There does not seem to exist a similar result in the case of blocks of defect 0. As a substitute, we have here the theorem:

**THEOREM 7:** *The classes of defect 0 in  $G$  form the basis of a subalgebra  $M$  of the center  $\Lambda^*$  of the modular group ring  $\Gamma^*$  of  $G$ . The number of blocks of defect 0 is equal to the rank of  $M^n$  for sufficiently large  $n$ .*

\* Part of the work on this and a following note was done while the author was a Fellow of the John Simon Guggenheim Memorial Foundation.

<sup>1</sup> "On the Arithmetic in a Group Ring," these PROCEEDINGS, 30, 109-114 (1944). This paper will be quoted as A G R.

<sup>2</sup> Brauer, R., *Am. Jour. Math.*, 67, 461-471 (1945).

<sup>3</sup> See for instance, Brauer, R., and Nesbitt, C., *Ann. Math.*, 42, 556-590 (1941).

<sup>4</sup> We denote the residue class field of the integers of  $K$  (mod  $\mathfrak{p}$ ) by  $K^*$  and the group ring of  $G$  with regard to  $K^*$  by  $\Gamma^*$ .

## THE RATE OF GROWTH OF ANALYTIC FUNCTIONS

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This note presents a theorem of Phragmén-Lindelöf type and indicates how it can be applied to establish and generalize results of N. Levinson<sup>1</sup> on the determination of the rate of growth of an analytic function along a line



from its growth on a sequence of points. Detailed proofs will be published elsewhere.

**THEOREM 1.** *Let  $\delta(r)$  be a continuous function such that  $0 \leq \delta(r) < 1/2$  and  $\int_0^\infty r^{-1}\delta(r)dr$  diverges. Let  $H(z)$  be analytic in  $x \geq 0$  and let it satisfy*

$$\log |H(re^{i\theta})| = o\{\omega(r)\}, \quad r \rightarrow \infty, \quad \omega(r) = \exp \left\{ \int_0^r s^{-1}\delta(s)ds \right\}.$$

*Let  $H(z)$  be bounded on the curve  $x = r\delta(r)$ . Then  $H(z)$  is bounded in  $x \geq r\delta(r)$ .*

The proof depends on the following lemma.

**LEMMA.** *Let  $u + iv = f(z)$  map the half plane  $x \geq 0$  on the region  $u \geq r\delta(r)$ ,  $r^2 = u^2 + v^2$ . Then for sufficiently large  $\rho$*

$$\max_{|\theta| \leq \pi/2} |f(\rho e^{i\theta})| \leq \eta(\rho),$$

*where  $s = \eta(\rho)$  is the inverse of  $\rho = C\omega(s)$ ,  $C$  being an appropriate constant.*

The lemma is a consequence of a theorem of Ahlfors on conformal mapping.<sup>2, 3</sup>

To prove the theorem, consider  $\psi(z) = H(f(z))$ . Then  $H(z)$  is analytic in  $x \geq 0$  and bounded on the imaginary axis, and

$$\log |\psi(re^{i\theta})| = o\{\omega(|f(re^{i\theta})|)\}.$$

By the lemma we have  $|f(re^{i\theta})| \leq \eta(r)$  for large  $r$ . Since  $\omega(r)$  is a non-decreasing function,  $\log |\psi(re^{i\theta})| = o\{\omega(\eta(r))\} = o(r)$ . By a well-known theorem of Phragmén and Lindelöf, this implies that  $\psi(z)$  is bounded in  $x \geq 0$ , and the theorem follows.

Now let  $\varphi(z)$  be analytic in  $x \geq 0$  and of exponential type there. Consider the problem of finding conditions under which

$$\limsup_{x \rightarrow \infty} x^{-1} \log |\varphi(x)| = \limsup_{x \rightarrow \infty} \lambda_n^{-1} \log |\varphi(\lambda_n)|, \quad (1)$$

where, for simplicity, we suppose here that the  $\lambda_n$  form a real increasing sequence and  $\lambda_{n+1} - \lambda_n \geq d > 0$ . We suppose that  $\{\lambda_n\}$  has a density, that is, that  $\lim_{n \rightarrow \infty} n/\lambda_n = D$ ,  $0 \leq D < \infty$ , or equivalently that  $\lim_{t \rightarrow \infty} t^{-1}\Lambda(t) \geq D$ , where  $\Lambda(t)$  denotes the number of  $\lambda_n$  not exceeding  $t$ . It was shown by V. Bernstein that (1) is true if

$$\limsup_{|y| \rightarrow \infty} |y|^{-1} \log |\varphi(iy)| = \pi L, \quad (2)$$

with  $D > L$ . Levinson<sup>1</sup> gave a simpler proof and gave sharper results for the case where (2) is strengthened and  $D = L$ . By the use of Theorem 1 we can extend Levinson's proof of Bernstein's theorem to prove not only Levinson's sharper theorems, but also more general results, including cases

where the hypothesis that  $\varphi(z)$  is of exponential type is considerably relaxed.

We first observe that, by replacing  $\varphi(z)$  by  $e^{-\alpha z}\varphi(z)$ , we may suppose that  $\limsup \lambda_n^{-1} \log |\varphi(\lambda_n)| = -\epsilon < 0$ . Let  $F(z)$  denote the canonical product with zeros  $\pm \lambda_n$ . With Levinson, we consider

$$g(z) = \sum_{n=1}^{\infty} \frac{\varphi(\lambda_n)F(z)}{(z - \lambda_n)F'(\lambda_n)}, \quad H(z) = \frac{\varphi(z) - g(z)}{F(z)}.$$

From known lemmas on  $F(z)$ , it follows that  $H(z)$  is analytic and of exponential type in  $x \geq 0$ . By imposing appropriate conditions on  $\{\lambda_n\}$  and on  $\varphi(iy)$ , the latter being a strengthened form of (2), we shall have

$$|H(re^{i\theta})| \leq A \exp \{ \pi L \cos \theta - r\delta(r) \} + B$$

for  $|\theta|$  near  $\pi/2$ , where  $A$  and  $B$  are constants and  $\delta(r)$  satisfies the conditions of Theorem 1. Theorem 1 then shows that  $H(x)$  is bounded, and so

$$\begin{aligned} |\varphi(x)| &\leq A|F(x)| + |g(x)|, \\ \limsup_{x \rightarrow \infty} x^{-1} \log |\varphi(x)| &\leq 0, \end{aligned}$$

and (1) will follow.

<sup>1</sup> Levinson, N., *Gap and Density Theorems*, New York, 1940, chap. 7.

<sup>2</sup> Nevanlinna, R., *Eindeutige Analytische Funktionen*, Berlin, 1936.

<sup>3</sup> A similar application of Ahlfors' lemma has been made by W. H. J. Fuchs, On the closure of  $\{e^{-t^{\alpha}}\}$ , *Proc. Cambridge Philos. Soc.*, 42, 91-105 (1946).

## A GENERALIZATION OF THE HOPF INVARIANT

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In this note we define a homomorphism of the  $n$ th homotopy group of the  $r$ -sphere  $S^r$  into the  $n$ th homotopy group of  $S^{3r-1}$  for  $n < 3r - 3$ . This homomorphism may be regarded as a generalization of the invariant defined by H. Hopf.<sup>1</sup> This homomorphism is used to construct new essential maps of spheres on spheres.

1. Let  $A$  and  $B$  be arcwise connected spaces,  $a_0$  and  $b_0$  points of  $A$  and  $B$ , respectively. Let  $A \vee B$  be the subset  $a_0 \times B \cup A \times b_0$  of the product  $A \times B$ ;  $A \vee B$  may be regarded as the union of the two spaces  $A$  and  $B$  with just one point in common. The homotopy groups of  $A \times B$  and  $A \vee B$  are related by

$$\pi_r(A \vee B) \approx \pi_r(A \times B) + \pi_{r+1}(A \times B, A \vee B) \quad (\text{direct sum}) \quad (1)$$

More precisely, in the homotopy sequence

$$\dots \rightarrow \pi_{r+1}(A \times B, A \vee B) \xrightarrow{\lambda} \pi_r(A \vee B) \xrightarrow{\mu} \pi_r(A \times B) \rightarrow \dots$$

$\mu$  is a homomorphism *onto* and  $\lambda$  an isomorphism *into*, while  $\pi_r(A \vee B)$  decomposes into the direct sum of the kernel of  $\mu$  (= the image of  $\lambda$ ), with a subgroup mapped isomorphically by  $\mu$ .

If  $A$  and  $B$  are spheres  $S^{p_1}$  and  $S^{p_2}$ , further results can be obtained. Let  $h_i$  be a map of the  $p_i$ -cell  $E^{p_i}$  which is topological on  $E^{p_i} - \dot{E}^{p_i}$  and maps  $\dot{E}^{p_i}$  into a point; and let  $h$  be the map of  $E^{p_1+p_2} = E^{p_1} \times E^{p_2}$  on  $S^{p_1} \times S^{p_2}$  defined by  $h(x_1, x_2) = (h_1[x_1], h_2[x_2])$ . Since  $h$  maps  $S^{p_1+p_2-1} = \dot{E}^{p_1+p_2}$  on  $S^{p_1} \vee S^{p_2}$ , it induces a homomorphism  $h^*: \pi_{n+1}(E^{p_1+p_2}, S^{p_1+p_2-1}) \rightarrow \pi_{n+1}(S^{p_1} \times S^{p_2}, S^{p_1} \vee S^{p_2})$ . By methods similar to those of Freudenthal<sup>2</sup> we can prove:

- (1)  $h^*$  is onto if  $n < p_1 + p_2 + \min(p_1, p_2) - 2$ ;
- (2)  $h^*$  is an isomorphism if  $n < p_1 + p_2 + \min(p_1, p_2) - 3$ .

It follows from (1) and known theorems that

$$\pi_n(S^{p_1} \vee S^{p_2}) \approx \pi_n(S^{p_1}) + \pi_n(S^{p_2}) + \pi_n(S^{p_1+p_2-1}) \quad (n < p_1 + p_2 + \min(p_1, p_2) - 3).$$

This generalizes a result of J. H. C. Whitehead.<sup>3</sup>

2. Let  $S^{r-1}$  be an equator of  $S^r$ ; identification of the points of  $S^{r-1}$  defines a mapping  $\phi: S^r \rightarrow S_1^r \vee S_2^r$ . Then  $\phi$  induces a homomorphism  $\phi^*: \pi_n(S_1^r) \rightarrow \pi_n(S_1^r \vee S_2^r)$ . If  $n < 3r - 3$  let  $\psi$  be the projection of  $\pi_n(S_1^r \vee S_2^r)$  on its direct summand  $\pi_n(S^{2r-1})$ ; then  $H = \psi\phi^*$  is a homomorphism of  $\pi_n(S^r)$  into  $\pi_n(S^{2r-1})$ .

Let  $f, g$  be mappings of  $S^p$  into  $S^q$ ,  $S^r$  into  $S^s$ , respectively. Represent  $S^{p+q+1}$  as the join of  $S^p$  with  $S^q$  and  $S^{r+s+1}$  as the join of  $S^r$  and  $S^s$ . Let  $f * g$  be the mapping of  $S^{p+q+1}$  into  $S^{r+s+1}$  which sends the segment  $\overline{xy}$  ( $x \in S^p, y \in S^q$ ) linearly into the segment  $\overline{f(x)g(y)}$ . The element of  $\pi_{p+q+1}(S^{r+s+1})$  represented by  $f * g$  depends only on the elements  $\alpha \in \pi_p(S^q)$ ,  $\beta \in \pi_r(S^s)$  represented by  $f$  and  $g$ ; call it  $\alpha * \beta$ . If  $g = s, g =$  the identity map, then  $\alpha * \beta$  is the  $(s+1)$ -fold *Einhängung*<sup>2</sup> of  $\alpha$ .

Let  $f: S^{p-1} \times S^{q-1} \rightarrow S^{r-1}$  be a mapping of type  $(\alpha, \beta)^1$ . Represent  $S^{p+q-1}$  as the join of  $S^{p-1}$  with  $S^{q-1}$ ; and let  $f^*$  be the mapping of  $S^{p+q-1}$  into  $S^r$  which maps the segment  $\overline{xy}$  ( $x \in S^{p-1}, y \in S^{q-1}$ ) linearly on the great semicircle from the north pole to the south pole of  $S^r$  which passes through the point  $f(x, y)$ . Then if  $\gamma$  is the element of  $\pi_{p+q-1}(S^r)$  represented by  $f^*$  and if  $p+q < 3r-2$ , then  $H(\gamma) = \alpha * \beta$ . It follows from a result of S. Eilenberg<sup>4</sup> that if  $\alpha$  is an element of  $\pi_{2r-1}(S^r)$  with Hopf invariant<sup>1</sup>  $h$ , then

$H(\alpha)$  is  $h$  times a generator of  $\pi_{2r-1}(S^{2r-1})$ . Thus  $H$  may be regarded as a generalization of the Hopf invariant.

Let  $R_{r-1}$  be the rotation group of  $S^{r-1}$ ,  $\pi$  the mapping of  $R_{r-1}$  into  $S^{r-1}$  which sends each rotation  $r$  into the image under  $r$  of a fixed point  $y_0 \in S^{r-1}$ . If  $p < 2r - 2$  and  $f$  is a mapping of  $S^{p-1}$  into  $R_{r-1}$ , then  $f$  defines a mapping  $F: S^{p-1} \times S^{r-1} \rightarrow S^{r-1}$  of type  $(\pi\alpha, 1)$ , where  $\alpha$  is the element of  $\pi_{p-1}(R_{r-1})$  represented by  $f$  and  $1$  is the element of  $\pi_{r-1}(S^{r-1})$  represented by the identity map. Then  $F$  determines an element  $\gamma$  of  $\pi_{p+r-1}(S^r)$  as in the preceding paragraph, with  $H(\gamma) =$  the  $r$ -fold *Einhangung*  $E\pi\alpha$  of  $\pi\alpha$ . Since  $p < 2r - 2$ ,  $E$  is an isomorphism<sup>2</sup> and it follows that if  $\pi\alpha \neq 0$ , then  $\gamma \neq 0$ . This result can be used to construct essential maps of  $S^n$  into  $S^r$  with  $n = 12, 14, 8k$  and  $16k + 2$ , and  $r = 6, 7, 4k$  and  $8k$ , respectively.

If  $r = 2, 4, 8$ , Hurewicz and Steenrod<sup>5</sup> have proved that  $\pi_n(S^r)$  is isomorphic with the direct sum  $\pi_n(S^{2r-1}) + \pi_{n-1}(S^{r-1})$ . This isomorphism determines a homomorphism  $H'$  of  $\pi_n(S^r)$  into  $\pi_n(S^{2r-1})$ . It is then easy to see that if  $n < 3r - 3$ , then  $H' = H$ .

3. Let  $X$  be an arcwise connected space,  $f$  a map of  $S^n$  into  $S^r$ , and  $g$  a map of  $S^r$  into  $X$ . The correspondence  $(f, g) \rightarrow gf$  defines an operation associating with  $\alpha \in \pi_n(S^r)$ ,  $\beta \in \pi_r(X)$  an element  $\beta \cdot \alpha \in \pi_n(X)$ . It is known that the left distributive law  $\beta \cdot (\alpha_1 + \alpha_2) = \beta \cdot \alpha_1 + \beta \cdot \alpha_2$  holds, while the corresponding right distributive law is in general false. Using the homomorphism  $H$  defined above, we can prove: if  $n < 3r - 3$ , then

$$(\beta_1 + \beta_2) \cdot \alpha = \beta_1 \cdot \alpha + \beta_2 \cdot \alpha + [\beta_1, \beta_2] \cdot H(\alpha)$$

where  $[\beta_1, \beta_2]$  is the product defined by J. H. C. Whitehead.<sup>3</sup>

<sup>1</sup> *Math. Ann.*, 104, 637-665 (1931); *Fund. Math.*, 25, 427-440 (1935).

<sup>2</sup> *Comp. Math.*, 5, 299-314 (1937).

<sup>3</sup> *Ann. Math.*, 42, 409-428 (1941).

<sup>4</sup> *Ibid.*, 41, 662-673 (1940).

<sup>5</sup> These PROCEEDINGS, 27, 60-64 (1941).

## ON THE STABILITY OF SYSTEMS OF DIFFERENTIAL EQUATIONS

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1. Consider the system of differential equations over the interval  $0 \leq t \leq \infty$

$$\frac{dx_i}{dt} = \sum_{j=1}^n a_{ij}x_j, \quad i = 1, \dots, n \quad (1)$$

and the perturbed system

$$\frac{dy_i}{dt} = \sum_{j=1}^n a_{ij}y_j + f_i(y_1, \dots, y_n, t). \quad (2)$$

We are interested in determining conditions on the  $f_i$  and  $a_{ij}(t)$  which will ensure that the solutions of (2) have properties similar to those of (1). The classical stability theory of Liapounoff,<sup>4</sup> where (1) is regarded as an approximation to (2), for particular  $f_i$ , is included in questions of this sort, as is also some fairly recent work of Cesari,<sup>5</sup> treating the case where the  $f_i$  are linear in the  $y_k$ . For the case of linear perturbation, a simple method of proof, sufficient also for the case of variable  $a_{ij}$ , was obtained by the author,<sup>1</sup> for the particular case of  $n$ th order linear differential equations. The same methods apply to linear systems.

Here, however, we are interested in a more general situation, and the purpose of this note is to indicate how Liapounoff's<sup>4</sup> and Cesari's<sup>5</sup> investigations are special cases of one more general result, which can be proved simply and directly by use of a classical method, the Picard iteration process.

For simplicity, we write the equation in vector form. Let  $x$  be the column vector with components  $x_i$ ,  $i = 1, \dots, n$ ,  $f(x, t)$  the column vector whose components are  $f_i(x_1, \dots, x_n, t)$ ,  $i = 1, \dots, n$ , and let  $A$  be the matrix  $a_{ij}$ ,  $i, j = 1, \dots, n$ .

Equation (1) becomes

$$\frac{dx}{dt} = Ax \quad (3)$$

and (2) becomes

$$\frac{dy}{dt} = Ay + f(y, t). \quad (4)$$

Furthermore, define

$$\|x\| = (\sum_i |x_i|^2)^{1/2}. \quad (5)$$

If  $\|x\|$  is bounded as  $t \rightarrow \infty$ , we say that the solution is bounded. The following results can now be stated:

**THEOREM 1:** *If all the solutions of (3) are bounded, all the solutions of (4) are bounded, provided:*

- (1)  $A$  is a constant matrix.
- (2)  $\|f(y, t)\| \leq c_m \phi(t)$  for  $\|y\| \leq m$ , and



$$(3) \int^{\infty} \phi(t) dt < \infty.$$

$$(4) \|f(y, t) - f(z, t)\| \leq d_m \psi(t) \|y - z\| \quad \text{for } \|y\| \leq m, \text{ and}$$

$$(5) \int^{\infty} \psi(t) dt < \infty.$$

THEOREM 2: *The same result holds if (1) is replaced by (1)':*

$$(1)' \quad \left| \int_0^t (\text{tr } A) dt \right| \leq c < \infty, \quad 0 \leq t \leq \infty. \quad (6)$$

Conditions (2) and (3) are satisfied if  $f(y, t)$  has all components of the form  $g(t)h(y)$ , where

$$\int^{\infty} g(t) dt < \infty \quad (7)$$

and  $h(y)$  has a continuous derivative with respect to  $y$  for  $0 \leq y < \infty$ .

Condition (3) is added to ensure that all solutions of the perturbed system are bounded. If it is a question of exhibiting perturbed solutions which are close to the original solution, the following result may be applied:

THEOREM 3: *If all solutions of (3) are bounded, there exist bounded solutions of (4), provided:*

(1)  $A$  is a constant matrix.

(2)  $f(y, t)$  is a continuous function of  $y$ , and

(3)  $\|f(y, t)\| \leq c_m \phi(t)$ ,  $\int^{\infty} \phi(t) dt < \infty$ .

Furthermore,  $y$  can be chosen to have the same value as  $x$  at a point,  $t_0$ , and for  $t \geq t_0(\epsilon)$ ,  $\|y - x\| \leq \epsilon$ .

As in Theorem 2, this result can be extended to variable  $A$ .

If a stronger requirement is imposed upon the matrix  $A$ , a more liberal perturbation is allowed. Thus, if we insist that the characteristic roots of

$$|A - \lambda I| = 0$$

all have negative real part, it is sufficient to assume merely that  $\phi(t)$  and  $\psi(t)$  are sufficiently small, depending upon  $A$ . It is more than enough that they tend to zero as  $t \rightarrow \infty$ . If we impose this restriction on  $A$ , and the condition on the  $f_i$  that they are power series in the  $y_k$  with constant coefficients, beginning with quadratic terms, we have a fundamental theorem of Liapounoff. It does not seem to have been previously noticed that the hypothesis of Cesari's theorem (essentially (2) of Theorem 1) can be weakened to a boundedness condition if the above restriction is made upon  $A$ .

We shall sketch the method of proof. The differential equation (4) is transformed into the integral equation

$$y = x + \int_0^t X(t) X^{-1}(t_1) f(y(t_1), t_1) dt_1, \quad (8)$$

where  $X(t)$  is the matrix solution of (3) satisfying  $X(0) = I$ .

If  $A$  is constant, the equation is even simpler, and becomes

$$y = x + \int_0^t X(t - t_1) f(y(t_1), t_1) dt_1. \quad (9)$$

The conditions imposed on  $f(y, t)$  and  $A$  are now sufficient to show convergence of the sequence

$$\begin{aligned} y_0 &= x \\ y_{n+1} &= x + \int_0^t X(t)X^{-1}(t_1)f(y_n(t_1), t_1)dt_1 \end{aligned} \quad (10)$$

using the classical methods.

Theorem 3 requires use of the Birkhoff-Kellogg<sup>2</sup> fixed point theorem applied to (8).

The Liapounoff result can be proved using the character of the solution of (3), for  $A$  restricted as above, and the quadratic character of the  $f_t$ .

2. Using the elementary methods mentioned previously, the following theorem can be obtained:

THEOREM 4: *All solutions of*

$$\frac{d^2x}{dt^2} = (A + B)x$$

*are bounded, provided*

(1)  $A$  is symmetric with negative characteristic roots.

(2)  $\|B\| = (\sum_{i,j} |b_{ij}|^2)^{1/2} \leq b$ .

(3)  $\int_0^\infty \left\| \frac{dB}{dt} \right\| dt < \infty$ .

The constant  $b$  depends upon  $A$ .

COROLLARY: *All solutions of*

$$y'' + (a^2 + \phi(x))y = 0$$

*are bounded, if*

(1)  $\int_0^\infty |\phi'(x)| dx < \infty$ .

(2)  $|\phi(x)| \leq b < a^2$ .

<sup>1</sup> Bellman, R., *Duke Math. Jour.*, 10, 643-648 (1943).

<sup>2</sup> Birkhoff, G. D., Kellogg, O. D., *Trans. Amer. Math. Soc.*, 23, 96-115 (1922).

<sup>3</sup> Cesari, L., *Ann. Scuola norm. super. Pisa*, 8, 131-148 (1939).

<sup>4</sup> Liapounoff, S., *Annales de Toulouse*, 9, 203-475 (1907).



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*ON NATURAL AUTO-ANTIBODIES AS EVIDENCED BY ANTI-  
VENIN IN SERUM AND LIVER EXTRACT OF THE GILA  
MONSTER\**

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In the course of experiments on the fertilizins and antifertilizins of sea-urchin eggs and sperm it was found<sup>1</sup> that an extract of eggs that had been deprived of their gelatinous surface coat was capable of agglutinating intact eggs and of forming a precipitate with the surface coat substance. Similar results have been obtained, although not consistently, with blood cells of mammals and with bacteria. On the basis of these and of various earlier reports in immunological literature that indicate a similar situation the view was proposed<sup>1, 2</sup> that all cells contain their own antibodies. The ability to demonstrate antigen-antibody like systems in a cell would, of course, depend upon use of appropriate extraction methods and upon the particular properties and amounts of the substances involved. Thus, for example, if surface substance were present in excess, an extraction procedure that involved destruction of the whole cell would permit interaction of the complementary surface and subsurface substances and the latter would not be found in the extract.

The finding of one antigen-antibody like system in a cell leads to the presumption that there may be others and to the view<sup>1, 2</sup> that such systems of complementary substances form the basis of cell structure. The synthesis of specific substances of large molecular size that comprise the structural elements of cells is thus conceived as occurring in a manner analogous to antibody formation. For the latter, there is now wide acceptance of the general views of Breinl and Haurowitz,<sup>3</sup> Alexander<sup>4</sup> and Mudd<sup>5</sup> involving an orienting influence of antigen, and Pauling<sup>6</sup> has formulated and experimentally supported<sup>7</sup> a specific mechanism based on the determination by antigen of the pattern of folding of polypeptide chains. This has been extended to the synthesis of specific substances and self-reproducing entities

(genes) in cells by Pauling and Delbrück<sup>8</sup> and Emerson.<sup>9, 10</sup> On a similar basis, schemes for the mode of action of genes and the possibility of inducing specific mutations by antibodies have been proposed and examined experimentally by Sturtevant<sup>11</sup> and Emerson.<sup>9, 10</sup>

The view<sup>1</sup> that all cells normally contain antibodies directed against certain of their constituents raises the possibility of deriving protective agents against pathogenic organisms directly by extraction of the organisms themselves rather than by the usual immunization procedures. The present author has made a number of attempts to obtain such protective auto-antibodies from pneumococci but these have, so far, been unsuccessful. Due to the factors mentioned above concerning possible extraction difficulties involved, the negative evidence cannot, as yet, be regarded as a sufficient basis for abandonment of the attempts. In the meantime, it appeared desirable to investigate other kinds of material from the same point of view. For this purpose a venomous reptile, the Gila monster, was selected. In general, venomous animals are resistant to homologous venom (see Calmette,<sup>12</sup> Noguchi,<sup>13</sup> Essex<sup>14</sup>) although the resistance is evidently<sup>15</sup> not absolute. Knowing this, many early workers have attempted to obtain auto-antivenins from the blood and organs, but with conflicting results<sup>12, 13, 14</sup>. More recently Rosenfeld and Glass<sup>16</sup> have shown that rattlesnake blood, although itself lethal for mice, neutralizes the hemorrhagic principle of the venom. Another report,<sup>17</sup> from the Serum Institute in Dorpat, Esthonia, is to the effect that viper serum loses its toxicity upon aging or heating, and is capable of neutralizing homologous venom. In the Gila monster, Cooke and Loeb<sup>18</sup> concluded that no antivenin was present in the serum. However, their data are not quite conclusive in that regard since, out of 17 mice that were given lethal doses of venom along with 1 ml. of serum, five survived. In the experiments reported here a definite protective action of serum and of liver extract is found.

*Material and Methods.*—Three Gila monsters of the species *Heloderma suspectum* were used. Two of these were injected with pilocarpine in order to induce copious secretion and exhaust the venom glands. The animals were each given an injection of 0.5 ml. of 1.3% pilocarpine on two consecutive days and the secretion collected each day shortly after the injection. The secretions were tested by intra-abdominal injection of mice. That collected on the first day proved lethal to mice in doses averaging 0.002 to 0.003 ml. while the second day's secretion was non-venomous in doses up to 0.2 ml. Soon after the second injection the animals were bled and venom glands, liver, pancreas, spleen and kidney removed. Later, serum was taken from the blood clots and the organs were washed and extracted with 10 volumes of saline by means of a Waring blender and centrifugation at 2000 g. for 30 minutes. Venom, serum and organ extracts were also obtained from the uninjected third animal to serve as a check on possible in-



TABLE 1  
TESTS OF VARIOUS SERA AND ORGAN EXTRACTS FOR ABILITY TO NEUTRALIZE  
HELODERMA VENOM

(Mice injected intra-abdominally with 1 ml. of mixtures containing 8 MLD of venom and dilutions of test material. Mixtures allowed to stand 15 minutes at room temperature. The asterisk designates tests of the toxicity of the material without venom.)

MATERIAL TESTED	AMOUNT, ML.	NO. OF MICE AT 4 DAYS	
		DEAD	ALIVE
*Serum of Heloderma #1 or #2	0.5	0	8
Serum of Heloderma #1 or #2	0.125 to 0.5	0	32
Serum of Heloderma #1 or #2	0.063	5	7
Serum of Heloderma #1 or #2	0.031	11	1
Serum of Heloderma #1 or #2	0.016	8	0
*Serum of Heloderma #3	0.5	0	4
Serum of Heloderma #3	0.25 and 0.5	0	24
Serum of Heloderma #3	0.125	6	6
Serum of Heloderma #3	0.063	8	4
Serum of Heloderma #3	0.031	10	2
Serum of Heloderma #3	0.016	3	1
*Serum of Rabbit	0.5	0	4
Serum of Rabbit	0.5	4	0
*Serum of Chuckwalla	0.5	0	4
Serum of Chuckwalla	0.063 to 0.5	20	0
Serum-Globulin of Heloderma #1	0.125 to 0.25	0	8
Serum-Globulin of Heloderma #1	0.063	3	1
Serum-Albumin of Heloderma #1	0.25	4	0
*Extract of Venom Gland of Heloderma #1	0.5	0	4
Extract of Venom Gland of Heloderma #1	0.5	7	1
Extract of Venom Gland of Heloderma #1	0.063 to 0.25	12	0
*Extract of Venom Gland of Heloderma #3	0.5	4	0
Extract of Venom Gland of Heloderma #3	0.125 to 0.5	12	0
*Extract of Liver of Heloderma #1	0.5	0	4
Extract of Liver of Heloderma #1	0.5	1	7
Extract of Liver of Heloderma #1	0.25	0	4
Extract of Liver of Heloderma #1	0.125	3	1
Extract of Liver of Heloderma #1	0.063	4	0
*Extract of Liver of Heloderma #3	0.5	0	4
Extract of Liver of Heloderma #3	0.5	7	1
Extract of Liver of Heloderma #3	0.25	5	3
Extract of Liver of Heloderma #3	0.125	5	3
Extract of Liver of Heloderma #3	0.063	7	1
*Extract of Pancreas of Heloderma #1	0.5	0	4
Extract of Pancreas of Heloderma #1	0.5	4	0
*Extract of Spleen of Heloderma #1	0.5	0	4
Extract of Spleen of Heloderma #1	0.5	4	0
*Extract of Kidney of Heloderma #1	0.5	0	4
Extract of Kidney of Heloderma #1	0.5	3	1

fluence of pilocarpine on the properties of these materials. In addition serum of the rabbit and of a non-venomous lizard, the chuckwalla (*Sauromalus ater*) were tested. All tests were made with white mice weighing about 28 g. They were kept for four days following injection. In no case did animals that survived the first day subsequently succumb and most deaths occurred within six hours. The venom was titrated in each set of experiments. The test dose employed in the experiments listed in table 1 contained 6 to 10 (av. 8) MLD. In these experiments venom and test material were mixed and kept at room temperature for 15 minutes before injection. Between sets of experiments the material was stored in the frozen state.

*Results.*—The site of synthesis of the venom in the Gila monster is not definitely known. On the supposition that it may be located in the venom gland, extracts of that organ were examined for the possible presence of anti-venin. The pilocarpine-injected animal was first investigated since the heavy secretion of venom induced in it offered a more favorable opportunity for obtaining antivenin. However, the results (see table 1) were negative. The efficacy of pilocarpine in exhausting the gland of venom is illustrated by the fact that 0.5 ml. of the extract alone proved non-toxic. On the other hand, the venom gland extract of the non-pilocarpine-injected animal (*Heloderma* #3) proved quite lethal. The latter extract was also tested in mixtures with venom, for possible mutual neutralizing action, but here, too, the results were negative.

Since the location of the site of synthesis of the venom is not definitely known, it seemed desirable to examine serum and extracts of other organs for possible venom-neutralizing action. As the results presented in table 1 show, the *Heloderma*'s serum and liver extract proved to be effective. In most of these tests both the venom and serum or extract employed were derived from the same individual. The protective action is exhibited by serum of both the pilocarpine-injected (#1 and #2) and the non-injected (#3) animals, and by liver-extract of a pilocarpine-injected animal (#1). Pilocarpine alone (unlisted tests) gives no protection. Complete protection against 8 MLD of venom is obtained with 0.125 to 0.25 ml. of serum and 50% protection with 0.063 to 0.125 ml. While the serum of the pilocarpine-injected animal has given somewhat better protection than that of the non-injected animal, more data would be required before any significance can be attached to the difference. In the case of the liver extracts the difference appears to be greater, but in view of the difficulty of maintaining identical treatment in preparation of the extract, it cannot as yet be concluded that liver from a non-pilocarpine-injected *Heloderma* is incapable of yielding much protective material. It may also be noted that the latter extract gives somewhat irregular results upon dilution. This may be due to a slow release of protective material from particulate matter in the extracts upon dilution or to some other unknown factor.

The tests with serum of the rabbit and of the chuckwalla revealed no neutralizing action on the part of these materials. Likewise the extracts of pancreas, spleen and kidney of *Heloderma* were found to be incapable of neutralizing any significant amount of venom.

Globulin and albumin fractions were prepared from *Heloderma* serum by precipitation with ammonium sulphate. The globulin fraction, adjusted to original serum concentration, was found to possess approximately the same neutralizing power as whole serum while the albumin exhibited no protective action.

Some tests (not listed in the table) were made in which the mice (16) received injections of *Heloderma* serum at various times after the injection of venom. With 0.125 ml. of serum complete protection was obtained at 3 to 6 minutes, partial protection at 10 to 12 minutes and no protection at 13 to 15 minutes.

*Discussion.*—The present results do not constitute direct evidence for the auto-antibody concept, as stated above, since for that purpose, it would be necessary to demonstrate the presence of auto-antivenin in the cells in which venom is synthesized. However, they do furnish some support for it, particularly since the expectation of finding an auto-antivenin somewhere in the animal was based on that view. The results may, then, be interpreted in the following manner. Venom and antivenin, in combination, are both assumed to be liberated into the blood stream from the organ, perhaps the liver, in which they are synthesized. The venom gland then effects a separation of the two so that venom accumulates in the gland from which it is later secreted, while the antivenin is left in the blood. Various methods of testing this and other similar interpretations suggest themselves and these will constitute the subject of further investigations that are planned along this line.

Several earlier investigations were mentioned in the previous articles<sup>1, 2</sup> as indicative of natural auto-antibodies. Since then others have appeared that point in the same general direction. Perhaps the most pertinent is the finding by Kidd and Friedewald<sup>19</sup> that normal rabbit serum reacts with a sedimentable constituent obtained in extracts of various organs of the same animal. This can be interpreted as due to the release into the blood of substances that are formed in connection with the sedimentable constituents of the tissues involved and that have configurations complementary to these constituents. On the basis of the auto-antibody concept one would, in fact, expect to be able to find complementary substances for all of the large molecular constituents of serum, located in the tissues where the latter are formed. Recent evidence,<sup>20</sup> pointing to the lymphocytes as the source of gamma-globulin, leads to the expectation of finding natural anti-globulin therein.

Auto-antibodies that are presumed to arise from an immunization process

are found in the serum of individuals having various diseases, such as: syphilis, infectious mononucleosis, yellow fever, acute hepatitis, malaria and atypical pneumonia.<sup>21</sup> In these cases it is generally believed that the pathogenic organism furnishes the foreign protein that serves to form a complete antigen with some constituent of the host tissue or that the organism possesses an antigen that is serologically similar to the host tissue constituent. On the basis of the natural auto-antibody concept a different interpretation may be offered. The infectious agent is assumed to release from the tissue undergoing destruction, pairs of mutually complementary substances, of which one is quickly removed from the circulation while the other remains there for a time. Thus, in syphilis, it would be presumed that the spirochaete produces a lipo-protein-splitting enzyme, such as has been reported<sup>22</sup> in other organisms, and that the lipid is removed from the blood leaving behind the complementary protein which is identified as the Wassermann reagin.

Naturally occurring substances that react with hormones or enzymes of the same animal have been described.<sup>23</sup> It seems possible that these, too, may represent auto-antibodies. There is also some evidence<sup>24, 25</sup> that antibacterial proteins such as lysozyme and diplococcin are derivable from the species of organism on which they act. It has, in fact, been suggested<sup>24</sup> that "Such enzymes, which in high concentration partly or completely lyse the organisms from which they are derived, are probably concerned with bacterial multiplication." Autolytic enzymes, such as that of the pneumococcus,<sup>26</sup> may belong to the same category.

While these various suggestions are largely of speculative nature, they have the advantage of being subject to experimental attack and offer the possibility of contributing to the analysis of cell growth and differentiation as well as to the practical problems of protection against infection and abnormal growth.

*Summary.*—Serum of *Heloderma* was found to be capable of neutralizing the venom of the same animal. Auto-antivenin was also found in extract of liver of a pilocarpine-injected *Heloderma* but not in extracts of venom gland, pancreas, spleen and kidney of this animal, nor was antivenin found in the serum of the rabbit and the chuckwalla. The antivenin was obtained in the globulin fraction of the *Heloderma* serum. The bearing of these results on the natural auto-antibody concept, previously proposed, is discussed and applications of this concept to various immunological and general biological problems are indicated.

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A NEW TYPE OF ISOLATING MECHANISM IN *DROSOPHILA*

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While working on the general problem of sexual isolation in the genus *Drosophila*, the writer discovered a mechanism which forms a very effective barrier against the exchange of genes between certain members of this genus. It was first detected in the cross *D. buzzatii* ♀ × *D. arizonensis* ♂, two members of the *mulleri* subgroup of species. It was later found to be operative between other members of this subgroup, as well as between species belonging to other groups. The phenomenon stems from a reaction of the vagina that almost immediately follows copulation, and may be designated the *insemination reaction*. Within a very short time after mating, the vagina begins to enlarge and soon takes on an edematous-like condition, increasing to three or four times the normal size of this organ in virgin females.

This change occurs in both intraspecific and interspecific crosses, but in intraspecific crosses (homogamic matings) the vagina returns to its normal size in the course of a few hours, while in interspecific crosses (heterogamic matings) it may remain swollen for several days and undergo deleterious changes which prevent it from carrying on its normal functions. In extreme cases, the reaction is so severe that eggs descending from the median oviduct may disintegrate in the vaginal cavity, or, if laid, they are not inseminated and are sometimes abnormal.

The *buzzatii-arizonensis* combination may be used to illustrate this reaction and its consequences. In previous publications from this laboratory, the cross *D. buzzatii* ♀ × *D. arizonensis* ♂ was designated as "incompatible." This term was employed in its usual sense to indicate that such test crosses do not result in the production of offspring. Recent studies on sexual isolation now reveal that the failure to produce hybrids in this cross is not due to the lack of insemination, but is the result of the insemination reaction that follows a successful copulation. When *buzzatii* females were crossed to *arizonensis* males in small mass cultures of ten females and ten males, 88% of the females were found to have been inseminated after an exposure of ninety-six hours, as shown by the presence of sperm in their ventral receptacles. We have tested this cross repeatedly but have never been able to obtain hybrids of any kind, either adults, pupae or larvae.

In studying this phenomenon, we have followed the plan of placing one virgin female with five males in a culture vial, and as soon as copulation had occurred the males and female were separated. Such females were dissected at given intervals of time and their reproductive tracts examined

for the presence of sperm and any changes that may have occurred in the vagina.

In homogamic matings the vagina begins to enlarge in about four minutes after copulation, and continues to enlarge until it reaches the maximum size at the end of about one hour. Within twenty-five to thirty minutes after copulation the vagina turns slightly opaque and continues to become more and more so up to the end of the second hour. It then begins to show signs of clearing, and within five or six hours its opacity has completely disappeared. It returns to its normal size and semi-transparent condition by the end of eight hours.

The behavior of the sperm following insemination is a matter of interest. Within five minutes after copulation the sperm begin moving along the ventral side of the vaginal cavity to the point of origin of the ventral receptacle and soon start entering that organ. They continue to pass into the receptacle until it may become packed with these elements. Since in species belonging to this subgroup, the sperm rarely ever enter the spermathecae, there is frequently an excess of sperm left in the swollen vagina. Eventually, these are expelled, together with the rest of the contents of the vagina, in the form of whitish droplets which are easily seen on the surface of the food and which may be removed to a slide and examined under the microscope.<sup>1</sup> Undoubtedly the voiding of the contents of the vagina accounts for the return of that organ to a normal condition by the end of the eighth hour. A majority of these females will, in due time, lay eggs and produce offspring without further inseminations.

In heterogamic matings the behavior of the sperm and the reaction of the vagina are at first similar to those occurring in intraspecific crosses, but certain significant differences soon appear. In the first place, the insemination reaction occurs somewhat more rapidly and the opacity of the vagina is more pronounced. Moreover, the sperm are slower in reaching the mouth of the ventral receptacle and do not begin entering that organ until about forty-five minutes after copulation. A large mass of sperm frequently collects about the opening to the receptacle, and the sperm have difficulty in their attempts to enter its lumen. A few sperm are usually present in the proximal end of the receptacle by the end of two hours, and by the end of three hours a few are at the distal end. While the quantity of sperm which finally reaches this position varies considerably, yet this amount is always very much less than is the case in homogamic matings. In one case, only two spermatozoa were present at the distal end, and in another only six. In practically all cases the sperm are restricted to the distal one-third or one-fourth of the ventral receptacle. In contrast to this condition, the receptacle always contained many sperm in homogamic matings and was frequently recorded as being "full" or "solid" with them.

In homogamic matings the vagina may soon return to a normal condi-

tion, as stated above, but in heterogamic matings its swollen condition may last for several days, accompanied by certain significant changes. One of the first differences noted is a change in color. At the end of the third hour after copulation the vagina turns slightly brownish and remains so up to about the twenty-fifth hour, when it often appears blackish under transmitted light. By forty hours the contents of the vagina become localized and sharply delimited, occupying the antero-dorsal part of the cavity. As time goes on this *reaction mass* becomes reduced in size and pear-shaped with the smaller end directed posteriorly. Further reduction may occur until its size does not constitute more than one-fourth the volume of the vagina. In some cases the reaction mass may undergo disintegration and entirely disappear, although usually traces of it can be detected. Even in such cases the vagina is left abnormal in appearance. In the *buzzatii-arizonensis* cross the reaction mass is usually present in females dissected on the seventh day after copulation.

Since only a part, and usually a very small part, of the sperm received by the female ever succeeds in entering the ventral receptacle, the question arises as to what becomes of those remaining in the vagina. Motile sperm are easily seen in this organ and have been detected in practically every case up to about the seventh hour after copulation. They have not been observed after this period, although the vagina of one female of the eighth hour contained a group of non-motile sperm located on the ventral side of its cavity. No evidence was obtained that the excess sperm had been expelled as in homogamic matings, but this remains as a possibility. It may be that these sperm are absorbed by the reproductive tract, as has been suggested for certain other insects.<sup>2</sup>

The sperm in the distal end of the ventral receptacle remain alive for at least 160 hours, and perhaps longer. There is, however, no evidence in the *buzzatii-arizonensis* cross that these sperm ever inseminate the eggs. The first egg discharged from the ovary in this series of dissections was forty-eight hours after copulation. It was just entering the median oviduct when first seen. One female dissected at fifty-six hours had laid five eggs, and two dissected at seventy-two hours had each laid one egg. These eggs were checked by the smear technique,<sup>3</sup> but none was found to contain sperm. The females had laid a considerable number of eggs by ninety-six hours, but most of these were transparent, like eggs laid by virgin females. A total of 105 of these eggs were checked for the presence of sperm and all were found not to have been inseminated. Some of the eggs break down and go to pieces in the vagina, and its cavity may become full of their debris. In one case, parts of three eggs were found in the vaginal cavity, and eggs in various degrees of disintegration were sometimes observed.

The intraspecific matings of nine different species of the *mulleri* subgroup have been examined for the presence of the insemination reaction and with-

out exception all showed that it occurred in them. In interspecific crosses between these species, the reaction was found to occur in all combinations in which copulation had been successful, irrespective of whether or not such matings had resulted in the production of hybrids. Successful copulations did not take place in a majority of the combinations tested, but in all crosses in which it did occur, the history of the insemination reaction was essentially the same as outlined above for the *buzzatii-arizonensis* cross. In the few cases in which hybrids were produced, the reproductive tract of the female had recovered sufficiently to allow some of the eggs to become inseminated. In most crosses, however, the number of hybrids produced was relatively small, and these were sometimes abnormal or else the zygotes never developed beyond the mid-larval stage. The most successful cross, in so far as the production of hybrids is concerned, was between the two most closely related forms, *D. mojavenensis* and *D. arizonensis*.

The hybrids from the cross *D. mojavenensis* ♀ × *D. arizonensis* ♂ are fertile, and pair mating tests show that approximately 75% of the females produce offspring, although the average number of individuals per culture is relatively low. In one experiment, six-day-old *mojavenensis* females were crossed to *arizonensis* males of the same age in small mass matings, and four days later the females were dissected and their seminal receptacles examined. It was found that 93% of these females had been inseminated, and of this number, the vagina was clear and normal in twenty-seven cases, clear and nearly normal in thirty-three others, and opaque and abnormal in the thirty-three remaining specimens. The ventral receptacles of all the females of groups one and two contained motile sperm, and these are the females that are responsible for the production of the hybrids. It is doubtful if the vagina in any of the females belonging to the third group would ever recover sufficiently to enable them to lay fertile eggs.

This brief account of the insemination reaction in the *mulleri* subgroup raises the question as to its possible occurrence in other species of the genus. In past studies on members of the *virilis* group we had, from time to time, observed this reaction in interspecific crosses, but had not realized its significance. With the knowledge gained in studying the *mulleri* series, we re-examined the *virilis* group and found that the reaction also occurs among members of that group. However, the insemination reaction is rather inconspicuous in homogamic matings and could be overlooked. The reaction is quite obvious in the heterogamic matings, although not so striking as in the *mulleri* series.

Mr. Marshall R. Wheeler has undertaken a study of all available species of *Drosophila* in the laboratory, with the view of determining the extent of the insemination reaction among other members of the genus. He studied the intraspecific crosses only and used the method given above for obtaining a timed series of stages. In the list given below, members of the *virilis* and



mulleri series were examined by the writer, all others were studied by Mr. Wheeler, to whom I am indebted for the privilege of including his results in advance of publication. The several species examined are given in their systematic order as outlined by Sturtevant.<sup>4</sup> The terms present and absent are used to indicate whether or not the insemination reaction was found to occur in the different species. The list is not complete, but gives all forms thus far examined.

Subgenus *Pholadoris*: present in *D. victoria*. Subgenus *Sophophora*: 1. saltans group, absent in *D. prosaltans*. 2. willistoni group, absent in *D. nebulosa*. 3. melanogaster group, absent in *D. melanogaster*. 4. obscura group, absent in *D. pseudoobscura*. Subgenus *Drosophila*: 1. quinaria group, present in *D. transversa*, *D. innubila*, *D. subpalustris*, *D. suboccidentalis* and *D. subquinaria*. 2. guttifera group, present in *D. guttifera*. 4. virilis group, present in *D. virilis*, *D. americana* and *D. texana*. 6. tripunctata group, absent in *D. tripunctata*. 7. funebris group, present in *D. funebris*. 8. repleta group, absent in *D. hydei*; present in *D. mercatorum*, *D. pararepleta*, *D. hexastigma* and *D. gibberosa*. 8a. mulleri subgroup, *D. anceps*, *D. aldrichi*, *D. arizonensis*, *D. buzzatii*, *D. hamatofila*, *D. mojavensis*, *D. peninsularis*, *D. mulleri* and *D. ritae*, present in all nine. 10. melanica group, present in *D. melanica*. 13. cardini group, absent in *D. cardini*. 14. immigrans group, present in *D. immigrans*. Miscellaneous forms, present in the two subspecies, *D. pallidipennis pallidipennis* and *D. p. centralis*.

This list shows that the insemination reaction was found to occur in twenty-eight of the thirty-five species examined. On the basis of this study, it seems safe to predict that it is present in all members of the quinaria and virilis groups and the mulleri subgroup, but probably absent in all members of the subgenus *Sophophora*. The repleta group presents an interesting situation. The reaction was found to be present in thirteen of the fourteen species examined, with *D. hydei* representing the exception. There is incomplete evidence that some of the forms closely related to *hydei* also do not have the reaction. If this turns out to be true, it will be possible to divide the large repleta group into two main divisions, one with and one without the reaction.

It is legitimate to speculate on the nature of the insemination reaction and its possible rôle in *Drosophila* speciation. It is well at first to restate briefly just what happens after copulation has taken place. The introduction of the semen with its contained spermatozoa into the vagina is followed almost immediately by a reaction of the mucous membrane which secretes a relatively large amount of fluid into the cavity. This is what brings about the characteristic swelling of the vagina. This membrane must be hypersensitive to the foreign protein, and, since the reaction occurs after the first copulation, its hypersensitiveness must be an inherited character. Whether the spermatozoa *per se*, or the semen, or both are responsible for calling forth the



reaction is a matter of considerable interest. We have observed, from time to time, that in some crosses the male had failed to deliver spermatozoa with the semen at the time of copulation, and yet the typical insemination reaction had occurred. The most careful examination of the freshly formed reaction mass in such cases failed to disclose the presence of sperm. Moreover, no evidence was found of any form of lysis that might have been responsible for the absence of sperm.<sup>6</sup>

A more convincing line of evidence that the sperm are not responsible for the reaction is seen in the results obtained in backcross matings of sterile  $F_1$  males. These males have slightly smaller testes than normal, and spermatozoa are never formed in them. The  $F_1$  males from the cross *arizonensis* ♀ × *mojavensis* ♂ are of this type, and they will sometimes mate with either *arizonensis* or *mojavensis* females. Their sperm-free semen always brings about the typical insemination reaction. It is, therefore, certain that the presence of sperm in the semen is not necessary for the induction of the reaction.

Another point of interest is whether the insemination reaction will be repeated in the vagina of a female which has mated a second time. The answer to this question will depend on the species. In the first place, in forms which do not show the reaction, copulation may occur two or more times, and in some species at relatively short intervals. In species which show a weak reaction (e.g., *virilis* group), the female may remate two or more times at intervals of several hours, and a reaction of about the same intensity as the first occurs after each mating. Finally, in species in which the reaction is severe the female may never remate, but if she does, it is only after a long period of time, but here too the reaction occurs, provided the copulation has been successful.

Three possible functions may be suggested for the insemination reaction. The first of these refers to its occurrence in homogamic matings. It may have the effect here of preparing the reproductive tract for the fertilization mechanism which is to follow. It should be pointed out, in this connection, that even in forms which show no visible reaction there still may be a change in the mucous membrane which has the same effect. Unfortunately, this suggestion cannot be tested experimentally.

A second possible rôle of the insemination reaction has a more direct bearing on the problem of speciation. In species which do not have this reaction, the female may copulate two or more times in intraspecific matings. In contrast to this, the female usually mates but once in species showing a strong reaction. In the first instance the male in the population could, and probably would, mate with the same female more than once, instead of fertilizing additional females. In the second instance the male would be forced to copulate with more than one female, if he were to mate more than a single time. This might be an advantage in a population with a restricted

number of males. If the insemination reaction arose as a mutation, irrespective of any selective value it might have to the species as a whole, it would spread throughout the population. This is because virgin females might be inseminated by males either with or without the factor. Females fertilized by males not carrying the factor may be remated by both kinds of males. However, a female inseminated by a male with the factor is much less likely to be remated. Hence, a female mated by a male not carrying the factor may have progeny by several males, but a female carrying the factor will ordinarily have progeny by only one male. This is a very interesting example of how a mutation, even though it be non-adaptive, would replace its alleles in the population.

A third rôle is revealed in interspecific crosses among forms which have the insemination reaction, for here it has the effect of either reducing the exchange of genes, or preventing such exchanges altogether. The *buzzatii-arizonensis* cross is an excellent example of the complete elimination of gene transfer. Sexual isolation in this cross is weak and copulation occurs almost as frequently (88%) as in the intraspecific cross (97%), and yet, as a result of the insemination reaction, the females are unable to produce offspring.

In conclusion, it should be pointed out that the widespread occurrence of this reaction in the genus would suggest that it may be present in forms other than *Drosophila*. It is possible that the results reported above may have some bearing on the controversial point regarding the effect of sperm injections on the development of the reproductive tract in immature mammals.<sup>6, 7</sup>

It is a pleasure to acknowledge the excellent assistance given by Miss Johanna Blumel who cultured and collected the flies used in the experiments, and by Mrs. Sarah B. Martin who made most of the dissections.

<sup>1</sup> In 1942 Dr. Wilson S. Stone and the writer observed this same habit in females of the virilis group.

<sup>2</sup> Cragg, F. W., *Ind. J. Med. Res.*, **8**, 32-39 (1920); **11**, 449-473 (1923).

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<sup>4</sup> Sturtevant, A. H., *Univ. of Tex. Publ.*, **4213**, 7-51 (1942).

<sup>5</sup> I am indebted to Dr. V. T. Schuhardt, of the department of Botany and Bacteriology, for valuable suggestions in preparing this account on the nature of the insemination reaction.

<sup>6</sup> Green-Armytage, V. B., *Proc. Roy. Soc. Med.*, **36**, 105 (1943).

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## HYBRIDIZATION BETWEEN *RANA PALUSTRIS* AND DIFFERENT GEOGRAPHICAL FORMS OF *RANA PIPIENS*

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In intraspecific crosses among different populations of *Rana pipiens* Schreber, hybridization leads to progressively greater embryonic defects with increasing latitudinal distance between the parent populations. Experiments have been performed with individuals from many localities in the eastern and central portions of the United States with the following results.<sup>1</sup> Normal or very slightly abnormal embryos are formed by crossing adults of these localities: Wisconsin  $\times$  Vermont; Vermont  $\times$  New Jersey; Vermont  $\times$  Oklahoma; New Jersey  $\times$  Louisiana; Louisiana  $\times$  Ocala, Florida; Ocala, Florida  $\times$  Englewood, Florida; Ocala, Florida  $\times$  Texas. In crosses between extreme northern and southern individuals, such as Vermont  $\times$  Florida or Wisconsin  $\times$  Texas, the hybrids are very abnormal. If the female parent is from the north and the male from the south, the hybrids exhibit marked retardation in rate of development, extreme head enlargement and pronounced circulatory system defects. In the reciprocal cross, southern female and northern male, retardation in rate, reduction in head size with frequent fusion of olfactory pits and eyes, and the absence of a mouth are the typical abnormalities. The mortality varies in different experiments but is usually high.

It has been established that crosses between *Rana pipiens* from Vermont and *Rana palustris* Le Conte result in healthy hybrids which can be carried through metamorphosis.<sup>2</sup> Therefore, a Vermont *pipiens* can be crossed successfully with a different species but not with southern members of its own species. This unusual situation indicated that the results of crosses between *Rana palustris* and different geographical forms of *Rana pipiens* would be of interest. To secure data on this subject the following crosses were made:

- \*Vermont *pipiens* ♀  $\times$  *palustris* ♂ (5)
- \**palustris* ♀  $\times$  Vermont *pipiens* ♂ (5)
- \*Wisconsin *pipiens* ♀  $\times$  *palustris* ♂ (2)
- \**palustris* ♀  $\times$  Wisconsin *pipiens* ♂ (1)
- \**palustris* ♀  $\times$  Oklahoma *pipiens* ♂ (1)
- Englewood, Florida *pipiens* ♀  $\times$  *palustris* ♂ (1)
- palustris* ♀  $\times$  Englewood, Florida *pipiens* ♂ (1)
- \*New Jersey *pipiens* ♀  $\times$  *palustris* ♂ (1)
- \**palustris* ♀  $\times$  New Jersey *pipiens* ♂ (1)
- \*Ocala, Florida *pipiens* ♀  $\times$  *palustris* ♂ (1)
- \**palustris* ♀  $\times$  Ocala, Florida *pipiens* ♂ (1)
- \*Texas *pipiens* ♀  $\times$  *palustris* ♂ (1)
- \**palustris* ♀  $\times$  Texas *pipiens* ♂ (1)

All of the *Rana palustris* used in these experiments were collected in Massachusetts. In each type of cross marked with an asterisk, transformed young were secured. The figure in parentheses following each cross gives the number of crosses made. In all experiments in which the hybrids were raised to metamorphosis, it was found that the pigment pattern of the young was intermediate to that of the parents. There was no evidence of differential mortality between hybrids and controls in any experiment. In the Englewood, Florida *pipiens* ♀ × *palustris* ♂ cross, some embryos were kept until they were 40 mm. in length. They were then discarded, though normal. In the reciprocal cross, the embryos were also normal but no attempt was made to raise them to metamorphosis.

Crosses between Vermont *pipiens* and *palustris* have been described previously.<sup>2</sup> The rate of development of the hybrids was intermediate between that of the parents. No evidence of morphological defect was observed at any time during development.

In crosses between Wisconsin *pipiens* and *palustris*, the hybrids exhibited no structural defects and some were raised to metamorphosis. The rate of development was not determined. The *burnsi* mutant<sup>3, 4</sup> of *pipiens* was used in these experiments.

The *palustris* ♀ × Oklahoma *pipiens* ♂ hybrids were structurally normal. The rate of development was not determined. Some of the hybrids were carried through metamorphosis.

The crosses between *palustris* and Englewood, Florida *pipiens* are of interest as the Florida individuals give very abnormal hybrids with northern populations of *pipiens*. The Englewood, Florida *pipiens* ♀ × *palustris* ♂ hybrids were structurally normal. The rate of development was not determined. As noted before, these embryos were kept until they were tadpoles 40 mm. in length. The *palustris* ♀ × Englewood, Florida *pipiens* ♂ hybrids were structurally normal and developed at the same tempo as the maternal controls. In a parallel experiment some sperm of this same Florida *pipiens* male was used to fertilize Vermont *pipiens* eggs. The resulting intraspecific hybrids had greatly enlarged anterior ends, defective circulatory systems, and were markedly retarded in rate of development. The contrast between normal interspecific and abnormal intraspecific hybrids was striking.

The same *palustris* female was used in experiments with New Jersey, Ocala, Florida and Texas *pipiens* males. The hybrids with the New Jersey and Florida males developed normally. There was a possible acceleration in rate of development in the *palustris* ♀ × New Jersey *pipiens* ♂ hybrids during neural fold stages but later these embryos were identical with the maternal controls. The *palustris* ♀ × Texas *pipiens* ♂ hybrids were retarded approximately ten per cent when the controls were in stage 20.<sup>5</sup> In addition the anterior end of these embryos showed a slight, but definite,

enlargement (this type of defect is observed in close latitudinal crosses of *Rana pipiens*). Embryos from these three crosses were raised to metamorphosis.

The cross, New Jersey *pipiens* ♀ × *palustris* ♂, resulted in morphologically normal embryos. The rate of development was not measured. The embryos were raised to metamorphosis.

The Ocala, Florida *pipiens* ♀ × *palustris* ♂ hybrids developed at a rate intermediate to that of the two parental species. These embryos were structurally normal and some were raised to metamorphosis. In a parallel experiment, eggs of this same female were fertilized with Vermont *pipiens* sperm. These intraspecific hybrids were retarded in development and had reduced anterior ends with fused olfactory pits.

The Texas *pipiens* ♀ × *palustris* ♂ hybrids were retarded (as were the reciprocal hybrids) and the anterior end appeared to be slightly reduced in size. Some of these embryos were raised to metamorphosis. Eggs from this same female were fertilized with New Jersey and Vermont *pipiens* sperm. Both groups of intraspecific hybrids were retarded in rate and were morphologically abnormal. The defects were most apparent in the Texas *pipiens* ♀ × Vermont *pipiens* ♂ embryos, many of which lacked a mouth. Once again gametes from the same individual were found to be more compatible with gametes of a different species than with those of the same species.

In summary, all of the crosses between *Rana palustris* and different populations of *Rana pipiens* (except Texas) give normal hybrids. The defects noticed in crosses with Texas *pipiens* are so slight that they would not have been detected without close study.

The inviability observed in some *Rana pipiens* intraspecific crosses is thought to be due to complementary lethal genomes.<sup>1</sup> If such is the case, the type of incompatibility encountered when two complementary lethal genomes are present in the same individual would probably not result if either of these genomes was combined with a third genome. In the latter case, the genomes might be compatible, or if incompatibility was present it would probably be due to interaction of factors entirely different from those leading to inviability in the original case. The data cited in this paper indicate that the *Rana palustris* genome does not combine, with a resulting lethal effect, with any of the *Rana pipiens* genomes tested.

A somewhat similar case, of inability of individuals to cross with members of the same species coupled with the ability to cross with members of a different species, has been reported in cotton.<sup>6,7</sup> The "crumpled" condition of the hybrids formed in certain crosses is due to the combined action of complementary lethal genes  $Cp_a$  and  $Cp_b$ .  $Cp_a$  is found in only one strain of *Gossypium arboreum*. In all other strains of this species, and in *Gossypium herbaceum*, this locus is represented by  $cp_a$ .  $Cp_b$  and  $cp_b$  occur with about



equal frequency in various strains of *Gossypium arboreum* and *Gossypium herbaceum*. An intraspecific cross, between an individual of *Gossypium arboreum* homozygous for  $cp_a$  and  $Cp_b$  with an individual of another strain homozygous for  $Cp_a$  and  $cp_b$ , would result in the "crumpled" condition. An interspecific cross involving either of these individuals and *Gossypium herbaceum* homozygous for  $cp_a$  and  $cp_b$  would result in normal progeny.

*Rana palustris* occurs in the eastern parts of the United States and Canada. Its range of distribution is entirely encompassed by the range of *Rana pipiens*. The Vermont, Wisconsin, New Jersey and Oklahoma localities mentioned in these experiments are within the ranges of both species. In the Louisiana, Texas, Ocala Florida and Englewood, Florida localities, *Rana pipiens* is found—but not *Rana palustris*. In the localities where the two species occur together, natural selection has not resulted in the development of hybrid inviability as an isolating mechanism. It is of interest to note that the one strain of *Rana pipiens* which shows even slight incompatibility with *Rana palustris* is found in a locality in which only the former is present. The primary isolating mechanisms between these two species are probably differences in spawning period combined with well-developed differences in ecological preference. The rôle of sexual selection is not known. The two species will mate in the laboratory.

*Summary.*—Although some intraspecific crosses in *Rana pipiens* lead to hybrid inviability, every strain of this species tested can be successfully hybridized with *Rana palustris*.

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<sup>1</sup> Moore, J. A., *Genetics*, 31, 304-326 (1946).

<sup>2</sup> Moore, J. A., *Jour. Exp. Zool.*, 86, 405-422 (1941).

<sup>3</sup> Moore, J. A., *Genetics*, 27, 408-416 (1942).

<sup>4</sup> Moore, J. A., *Jour. Heredity*, 34, 2-7 (1943).

<sup>5</sup> These stages are described in: Pollister, A. W., and Moore, J. A., *Anat. Rec.*, 68, 489-496 (1937).

<sup>6</sup> Hutchinson, J. B., *Jour. Genetics*, 25, 281-291 (1932).

<sup>7</sup> Silow, R. A., *Ibid.*, 42, 259-358 (1941).

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## ON A THEOREM OF VON NEUMANN

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J. von Neumann pointed out in a recent talk at Harvard the need for an elementary proof of the following theorem, which was originally proved by him using the Brouwer fixed-point theorem.<sup>1, 2</sup> The present note supplies such a proof.

**THEOREM.** *Let  $a_{ij}$  and  $b_{ij}$  be two rectangular matrices ( $i = 1, \dots, n$ ;  $j = 1, \dots, m$ ) such that  $a_{ij} > 0$  for all  $i, j$ . Then there exists a unique  $\lambda$  and vectors  $x = (x_1, \dots, x_m)$ ,  $y = (y_1, \dots, y_n)$  subject to  $x_j \geq 0$ ,  $y_i \geq 0$ ,  $\sum_1^m x_j = 1$  and  $\sum_1^n y_i = 1$ , such that*

$$\lambda \sum_{j=1}^m a_{ij} x_j \geq \sum_{j=1}^m b_{ij} x_j, \quad i = 1, \dots, n, \quad (1)$$

$$\lambda \sum_{i=1}^n a_{ij} y_i \leq \sum_{i=1}^n b_{ij} y_i, \quad j = 1, \dots, m. \quad (2)$$

Let  $X$  be the set of  $m$ -dimensional vectors  $x = (x_1, \dots, x_m)$  such that  $x_j \geq 0$ ,  $j = 1, \dots, m$  and  $\sum_1^m x_j = 1$ , and let  $Y$  be the corresponding set of  $n$ -dimensional vectors. Let (2') be the inequalities (2) with  $\lambda$  replaced by  $\mu$ . It is evident that  $\lambda$  and  $x$  in  $X$  satisfying (1) exist, and that  $\mu$  and  $y$  in  $Y$  satisfying (2') exist. Moreover (1) and (2') yield

$$\mu \sum_i \sum_j a_{ij} y_i x_j \leq \sum_i \sum_j b_{ij} y_i x_j \leq \lambda \sum_i \sum_j a_{ij} y_i x_j \quad (3)$$

In particular,  $\mu \leq \lambda$ , so that the values of  $\lambda$  which can be used in (1) are bounded below, and since  $X$  is compact, the greatest lower bound  $\lambda_0$  can be used in (1) for a certain vector  $x^0$ . Similarly the least upper bound  $\mu_0$  can be used in (2') for a certain vector  $y^0$ . And  $\mu_0 \leq \lambda_0$ .

The theorem deals with those  $\lambda$  of (1) which are also  $\mu$  of (2'). Clearly the only  $\lambda$  having this property is  $\lambda = \lambda_0$ , and even this will not do if  $\mu_0 < \lambda_0$ . Hence we must prove that  $\mu_0 = \lambda_0$ .

The proof of the theorem is an induction on  $m + n$ . If  $m + n = 2$ , the theorem is trivial. In the general case, if equality occurs in (1) for all  $i = 1, \dots, n$  when  $\lambda = \lambda_0$ ,  $x = x^0$  and also in (2') for all  $j = 1, \dots, m$  when  $\mu = \mu_0$ ,  $y = y^0$ , then equality occurs in (3), so that  $\mu_0 = \lambda_0$  and the theorem is established. It remains only to consider the case when strict inequality holds at least once. We may suppose therefore that

$$\begin{aligned} \lambda_0 \sum_j a_{ij} x_j^0 &= \sum_j b_{ij} x_j^0, \quad i = 1, \dots, n_1, \\ \lambda_0 \sum_j a_{ij} x_j^0 &> \sum_j b_{ij} x_j^0, \quad i = n_1 + 1, \dots, n. \end{aligned} \quad (4)$$

Let  $\lambda_1$  and  $\mu_1$  be the extreme values of  $\lambda$  and  $\mu$  in (1) and (2') for the reduced matrices ( $i = 1, \dots, n_1$ ;  $j = 1, \dots, m$ ). Then

$$\lambda_1 \leq \lambda_0, \quad \mu_1 \leq \mu_0. \quad (5)$$

For, every  $\lambda$  and  $x = (x_1, \dots, x_m)$  satisfying (1) for  $i = 1, \dots, n$  also satisfies (1) for the reduced set  $i = 1, \dots, n_1$ . And every  $\mu$  and  $y = (y_1, \dots, y_{n_1})$  satisfying the reduced (2') (with  $n$  replaced by  $n_1$  in the sums) also satisfies the original (2') if  $y$  is extended to  $y = (y_1, \dots, y_{n_1}, 0, \dots, 0)$ . We assert that in fact  $\lambda_1 = \lambda_0$ . Suppose, on the contrary, that  $\lambda_1 < \lambda_0$ , and that  $\lambda_1$  is assumed at  $x = x'$ , i.e., that

$$\lambda_1 \sum_j a_{ij} x'_j \geq \sum_j b_{ij} x'_j, \quad i = 1, \dots, n_1. \quad (6)$$

Then if  $x = \alpha x^0 + (1 - \alpha)x'$ , where  $0 < \alpha < 1$  (note that  $x \in X$ ), we have from (4) and (6) that

$$\lambda_0 \sum_j a_{ij} x_j > \sum_j b_{ij} x_j, \quad i = 1, \dots, n_1, \quad (7)$$

and it follows from (4) and the continuity of the expressions involved that (7) remains true for  $i = n_1 + 1, \dots, n$  if  $\alpha$  is small enough. Hence  $\lambda_0$  is not the extreme value for (1), a contradiction. Thus  $\lambda_0 = \lambda_1$ . But  $\lambda_1 = \mu_1$  by the inductive hypothesis,  $\lambda_1 \leq \mu_0$  by (5) and  $\mu_0 \leq \lambda_0$  from (3). Therefore,  $\mu_0 = \lambda_0$  and the theorem has been proved.

If  $\mu = \mu_0 = \lambda_0 = \lambda$ ,  $x = x^0$ ,  $y = y^0$ , then (3) becomes an equality. Therefore, if strict inequality holds in (1) at  $i = i_0$  then  $y_{i_0}^0 = 0$ , and similarly if strict inequality holds in (2'). If the matrices are square and  $b_{ii}$  is the unit matrix, it is evident from (1) that  $\lambda_0 (= \mu_0) > 0$ , so that (2') implies that  $y_i^0 > 0$  and (1) is composed of equalities. But then  $x_j^0 > 0$  and (2') is composed of equalities. Thus we deduced as a corollary the existence of a positive proper value and the associated positive proper vectors for a positive matrix and its transpose.

For the application of the theorem to the theory of production, see reference 1. The special case where all  $a_{ij} = 1$  is important in the theory

of games. For this see reference 3, pp. 154–155, where another proof of this special case is also referred to.

<sup>1</sup> von Neumann, J., "Über ein ökonomisches Gleichungssystem, etc.," *Ergebnisse eines Mathematischen Kolloquiums*, **8**, 73–83 (1937).

<sup>2</sup> Kakutani, S., "A Generalization of Brouwer's Fixed Point Theorem," *Duke Math. Jour.*, **8**, 457–459 (1941).

<sup>3</sup> von Neumann, J., and Morgenstern, O., "Theory of Games and Economic Behavior," Princeton University Press (1944).

## ON BLOCKS OF CHARACTERS OF GROUPS OF FINITE ORDER, II

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1. The first part of this investigation appeared in these PROCEEDINGS, June, 1946, p. 182.<sup>1</sup> In this note, we shall apply our results to a study of the (generalized) decomposition numbers<sup>2</sup> of a group  $G$  of finite order  $g$  and of the arithmetic in the group ring  $\Gamma$  of  $G$ .

Let again  $p$  be a fixed rational prime number. Select a full system  $\Pi$  of elements  $\pi_0 = 1, \pi_1, \pi_2, \pi_3, \dots$  of orders  $1, p, p^2, \dots$  such that every element of an order  $p^\alpha$  of  $G$  is conjugate in  $G$  to exactly one element  $\pi_i$  of  $\Pi$ . Denote by  $N_i$  the centralizer of  $\pi_i$  in  $G$ . A full system  $\Sigma$  of elements of  $G$  representing the different classes of conjugate elements can be obtained in the following manner: Let  $\sigma_1^{(i)}, \sigma_2^{(i)}, \dots$  represent the different  $p$ -regular classes of conjugate elements of  $N_i$ . Then  $\Sigma$  consists of the elements  $\pi_i \sigma_1^{(i)}, \pi_i \sigma_2^{(i)}, \dots$  for  $i = 0, 1, 2, \dots$ .

2.<sup>3</sup> If  $\zeta_1, \zeta_2, \dots, \zeta_k$  are the ordinary irreducible characters of  $G$ , and if  $\varphi_1^i, \varphi_2^i, \dots$  are the modular irreducible characters of  $N_i$ , then for every  $p$ -regular element  $\sigma$  of  $N_i$ , we have a formula

$$\zeta_\mu(\pi_i \sigma) = \sum_\nu d_{\mu\nu}^i \varphi_\nu^i(\sigma) \quad (1)$$

where the  $d_{\mu\nu}^i$  are algebraic integers, the *decomposition numbers*, which are independent of  $\sigma$ . This formula yields a representation of the matrix  $Z$  of the ordinary characters of  $G$  as a product of two square matrices  $D$  and  $\Phi$

$$Z = D\Phi. \quad (2)$$

We have to set  $\sigma = \sigma_j^{(i)}$ ,  $Z = (\zeta_\mu(\pi_i \sigma_j^{(i)}))$ , where  $\mu$  is the row index while every column corresponds to an element  $\sigma_j^{(i)}$ ,  $i = 0, 1, 2, \dots; j = 1, 2, \dots, k_i$ , where  $k_i$  is the number of  $p$ -regular classes of  $N_i$ . Similarly, in  $D =$

$(d_{\mu\nu}^i)$ , the rows correspond to the characters  $\zeta_\mu$  and the columns to the modular characters  $\varphi_\nu^i$ , of the different  $N_i$ . Finally, if the rows and columns are arranged suitably,

$$\Phi = \begin{pmatrix} (\varphi_\nu^0(\sigma_j^{(0)})) & 0 & \cdots \\ 0 & (\varphi_\nu^1(\sigma_j^{(1)})) & \cdots \\ \cdots & \cdots & \cdots \end{pmatrix} \quad (3)$$

where, in each partial matrix  $(\varphi_\nu^i(\sigma_j^{(i)}))$  in the main diagonal, the row index is  $\nu$  and the column index is  $j$ .

The degree of all three matrices in (2) is equal to the number  $k$  of conjugate classes of  $G$ ,

$$k = k_0 + k_1 + \cdots.$$

The square of the determinant of  $D$  is a power of  $p$  while the determinant of  $\Phi$  is relatively prime to  $p$ . The formulae (1) show that in order to know the ordinary characters of  $G$ , it is sufficient to know the modular characters  $\varphi_\nu^i$  of all the  $N_i$  including  $N_0 = G$ , and the decomposition numbers  $d_{\mu\nu}^i$ . Actually, the product of the column  $(i, \nu)$  of  $D$  with the conjugate complex of the column  $(i', \nu')$  is 0 for  $i \neq i'$  and the Cartan invariant  $c_{\nu, \nu'}^i$  of  $N_i$  for  $i = i'$ . While this  $c_{\nu, \nu'}^i$  can be expressed in terms of the  $\varphi_\nu^i$ , this does not enable us to express the decomposition numbers in terms of the  $\varphi_\nu^i$ .

We shall say that for fixed  $i$  the elements  $\pi_i \sigma_j^{(i)}$  of  $G$  belong to the  $i$ th section.

3. In the notation of I, theorem 1, we take  $H$  as the group generated by  $\sigma_i$ , and  $M = \mathfrak{G}(H) = N_i$ . The following result can be proved (with considerable difficulty):

**THEOREM 1:** *If the modular character  $\varphi_\nu^i$  of  $N_i$  belongs to a block  $\tilde{B}_\sigma$  of  $N_i$ , then  $d_{\mu\nu}^i$  can be different from 0 only for ordinary characters of  $G$  which belong to the block  $B_\sigma$  of  $G$  determined by  $\tilde{B}_\sigma$ .*

This implies that in each column of  $D$  we have zero except in the rows corresponding to the  $\zeta_\mu$  belonging to one block  $B_\sigma$  of  $G$ . It follows that, if the rows and columns of  $D$  are taken in a suitable order,  $D$  breaks up completely into  $l$  matrices  $T_1, T_2, \dots, T_l$ , each  $T_\tau$  corresponding to one block  $B_\tau$ , ( $\tau = 1, 2, \dots, l$ ). Since  $\det D \neq 0$ , each  $T_\tau$  must necessarily be a square matrix, of degree  $x_\tau$ , where  $x_\tau$  is the number of ordinary characters in  $B_\tau$ . The arrangement of the columns of  $D$  here will in general not be the same as that used in (2).

Originally, only the ordinary characters  $\zeta_\mu$  of  $G$  and the modular characters  $\varphi_\nu^0$  of  $G$  itself were distributed into blocks  $B_\tau$ . It is now natural to count  $\varphi_\nu^i$ ,  $i \geq 0$ , as a character of  $B_\tau$ , if  $\varphi_\nu^i$  belongs to a block  $\tilde{B}_\sigma$  of  $N_i$  which determines  $B_\tau$  in the sense of I. Then  $B_\tau$  consists of  $x_\tau$  ordinary characters  $\zeta_\mu$  and  $x_\tau$  modular characters  $\varphi_\nu^i$ . In our notation,  $y_\tau$  of these characters have the upper index  $i = 0$ . These are the modular characters of  $G$ , the other  $\varphi_\nu^i$  are the modular characters of the groups  $N_i$ .



As a corollary to theorem 1, we have the following refinement of some of the orthogonality relations for group characters.

**THEOREM 2:** *If the elements  $\rho$  and  $\sigma$  of  $G$  belong to different sections of  $G$ , then*

$$\sum_{\mu}' \zeta_{\mu}(\rho) \zeta_{\mu}(\sigma) = 0$$

when in the sum  $\zeta_{\mu}$  ranges over all the characters of  $G$  belonging to a fixed block  $B_{\tau}$ .<sup>4</sup>

4. We state without proof the following results which are connected with theorem 1.

**THEOREM 3:** *Let  $B_{\tau}$  be a block of  $G$ , and  $D_{\tau}$  its defect group. If no element of  $D_{\tau}$  is conjugate to  $\pi_1$ , then  $\zeta_{\mu}(\rho) = 0$  for all characters  $\zeta_{\mu}$  of  $B_{\tau}$  and all elements of the section of  $\pi_1$ .<sup>5</sup>*

**THEOREM 4:** *If  $B_{\tau}$  is a block of defect  $d_{\tau}$  with the defect group  $D_{\tau}$ , there exist blocks  $\tilde{B}_{\sigma}$  of defect  $d_{\tau}$  of  $N_1$  which determine  $B_{\tau}$ , if and only if  $\pi_1$  is conjugate in  $G$  to an invariant element of  $D_{\tau}$ . If  $\pi_1$  is conjugate to an invariant element of  $D_{\tau}$ , we can choose the block  $\tilde{B}_{\sigma}$  of defect  $d_{\tau}$  of  $N_1$  in such a manner that it determines the block  $B_{\tau}$  of  $G$ , and that for every  $\zeta_{\mu}$  in  $B_{\tau}$  there exists a  $\varphi_{\mu}^i$  in  $\tilde{B}_{\sigma}$  such that  $d_{\mu}^i \neq 0$ .*

Let  $p^a$  be the exact exponent to which  $p$  divides  $g$ ,

$$g = p^a g', \quad (p, g') = 1$$

If  $\mathfrak{p}_0$  is a prime ideal divisor of  $p$  in the field of characters, and if the degree  $\varkappa_{\mu}$  of  $\zeta_{\mu}$  contains  $p$  to the exact exponent  $a - d_{\tau} + \epsilon$ , ( $\epsilon \geq 0$ ), we may even state in theorem 4 that for a suitable  $\varphi_{\mu}^i$  in  $B_{\tau}$  we have

$$d_{\mu}^i \not\equiv 0 \pmod{p^* \mathfrak{p}_0}. \quad (4)$$

**THEOREM 5:** *If the block  $\tilde{B}_{\sigma}$  of  $N_1$  determines the block  $B_{\tau}$  of  $G$ , the defect of  $\tilde{B}_{\sigma}$  is at most equal to  $l$ , where  $p^l$  is the order of a maximal  $p$ -subgroup of  $N_1$  which is conjugate in  $G$  to a subgroup of the defect group  $D_{\tau}$  of  $B_{\tau}$ . If the degree  $\varkappa_{\mu}$  of the character  $\zeta_{\mu}$  of  $B_{\tau}$  is not divisible by  $p^{a-d+1}$  where  $d$  is the defect of  $B_{\tau}$ , there exists a character  $\varphi_{\mu}^i$  of  $N_1$  which belongs to a block of  $N_1$  of defect  $l$  and for which  $d_{\mu}^i$  is not divisible by  $\mathfrak{p}_0$ .*

**THEOREM 6:** *If  $p^a$  is the maximal order of elements of the defect group  $D_{\tau}$  of  $B_{\tau}$ , then for all  $\zeta_{\mu}$  in  $B_{\tau}$ , the numbers  $d_{\mu}^i$  belong to the field of the  $p^a$ -th roots of unity. The characters  $\zeta_{\mu}$  of  $B_{\tau}$  belong to the field of the  $(p^a g')$ -th roots of unity.*

**THEOREM 7:** *If  $p \neq 2$ , and if  $B_{\tau}$  contains  $y_{\tau}$  modular characters of  $G$ , then at least  $y_{\tau}$  of the ordinary characters  $\zeta_{\mu}$  of  $B_{\tau}$  are  $p$ -rational, that is, they lie in the field of the  $g'$ -th roots of unity,  $(g', p) = 1$ .*

In fairly general cases, the exact number of  $p$ -rational characters in  $B_{\tau}$  is equal to  $y_{\tau}$ .

For  $p = 2$ , a result similar to theorem<sup>7</sup> can be obtained which is more complicated, and shall not be stated here.

5. The previous results make it possible to prove the following theorem:

**THEOREM 8:** *A block  $B$  of defect  $d$  contains at most  $p^{d(d+1)/2}$  ordinary characters.*

It is probable that the bound  $p^{d(d+1)/2}$  here can be replaced by  $p^d$ , but I have been able to prove this stronger result only for  $d = 0, 1, 2$ .

Theorem 8 implies that if the order  $g$  of a group is divisible by the prime number  $p$  to the exact exponent  $a$ , and if  $G$  contains  $q$  classes of conjugate elements whose order is prime to  $p$  but whose normalizer has an order divisible by  $p^a$ , then at most  $qp^{a(a+1)/2}$  of the degrees of ordinary irreducible representations of  $G$  are relatively prime to  $p$ .

6. As in I, let  $K$  be an algebraic number field in which all the simple constituents of the semisimple algebra  $\Gamma$  split completely. Denote by  $\mathfrak{p}$  a fixed prime ideal divisor of  $p$  in  $K$ . The ideal  $(\mathfrak{p})$  generated by  $\mathfrak{p}$  in the ring of integers  $J$  of  $\Gamma$  can be represented as a direct intersection<sup>6</sup>

$$(\mathfrak{p}) = \mathfrak{M}_1 \cap \mathfrak{M}_2 \cap \dots \cap \mathfrak{M}_r$$

of ideals of  $J$ , such that no  $\mathfrak{M}_i$  possesses a proper representation as direct intersection. There exists a  $(1-1)$  correspondence between these "block components"  $\mathfrak{M}_i$  of  $(\mathfrak{p})$  and the blocks  $B_i$  of characters of  $G$  (for  $p$ ). In particular, the number  $y_i$  of modular characters of  $G$  is equal to the number of prime ideals  $\mathfrak{P}$  of  $J$  dividing  $(\mathfrak{p})$ . Now, theorem 8 implies

**THEOREM 9:** *No block component of  $(\mathfrak{p})$  in  $J$  is divisible by more than  $p^{a(a+1)/2}$  prime ideals of  $J$  where  $p^a$  denotes again the highest power of  $p$  dividing  $g$ .*

The  $y_i^2$  coefficients of the Cartan matrix  $C_i$  of the block  $B_i$  describe, to a certain extent, the mutual relationship between the  $y_i$  prime ideal divisors  $\mathfrak{P}$  of  $\mathfrak{M}_i$ . They represent interesting arithmetical invariants. Here,  $C_i$  is a symmetric matrix with integral rational coefficients. We can form the corresponding quadratic form  $Q$ . Now our results yield

**THEOREM 10:** *To given  $p$  and given defect  $d$ , there exist only a finite number of classes of quadratic forms to which the Cartan form  $Q$  of a block of defect  $d$  can belong (for an arbitrary group  $G$  of finite order).*

We also quote the following results which can be proved directly without great difficulty.

**THEOREM 11:** *If the defect of the block  $B_i$  is positive, the Cartan form  $Q$  does not represent the number 1. More generally,  $Q$  does not represent (integrally) a form of determinant 1.*

If  $B_i$  has the defect 0, then  $C_i$  is of degree 1, and  $Q$  is the quadratic form  $x^2$ .

7. It may be remarked that blocks of defect 1 can now be discussed rather completely. The results obtained earlier for the characters of groups

of an order  $g = pg'$ ,  $(p, g') = 1$  appear as special cases of properties of characters of blocks of defects 0 and 1.<sup>7</sup>

Finally, it may be mentioned as a conjecture that it appears probable that for a given  $p$  and  $d$ , only a finite number of matrices exist which can occur as Cartan matrices  $C_r$  of blocks of defect  $d$ .

<sup>1</sup> The first part will be quoted as I.

<sup>2</sup> Cf. Brauer, R., *Ann. Math.*, **42**, 926-935 (1941).

<sup>3</sup> For the results quoted in this section, cf. the paper mentioned in <sup>2</sup>.

<sup>4</sup> In the case that  $p$  belongs to the section of the 1-element, this result has already been obtained in Brauer, R., and Nesbitt, C., *University of Toronto Studies, Math. Ser.*, No. 4, theorem VIII (1937).

<sup>5</sup> This generalizes a result obtained in Brauer, R., and Nesbitt, C., *Ann. Math.* **42**, 556-590 (1941) for blocks of defect 0.

<sup>6</sup> Cf. Brauer, R., these PROCEEDINGS, **30**, 109-114 (1944), in particular, equation (2).

<sup>7</sup> Cf. Brauer, R., these PROCEEDINGS, **25**, 290-295 (1939), and *Ann. Math.* **42**, 936-958 (1941).

I take this occasion to mention the following corrections in the first of these papers: In theorem III, the assumption should read  $n < (2p + 7)/3$ . The left side of equation (4) should read  $r_{\rho}l_{\mu} + r_{\mu}l_{\rho}$ . For the results of the last paragraph of section 3, it is necessary to assume that a suitable splitting field is used.

## EFFECTS OF EXPOSURE TO ULTRA-VIOLET LIGHT ON HUMAN DARK ADAPTATION\*

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Previously it has been shown that the course of dark adaptation of the eye of the baby chick can be altered by addition of ultra-violet radiation between 290 and 365  $m\mu$  to the visible white light of a mercury vapor lamp during preexposure.<sup>1</sup> Exposure to wave-lengths longer than 365  $m\mu$  results in uniform dark adaptation curves, all curves reaching the same final threshold level. The addition of ultra-violet below 365  $m\mu$  retards complete adaptation, raising the final threshold considerably above the normal. Extension of the ultra-violet range to about 355  $m\mu$  causes an increase of 0.3 log unit, to 315  $m\mu$  an increase of 0.6 log unit, and to 290  $m\mu$  an increase of 1.1 log units in the final threshold level.

In the baby chick, as in all newly born animals, the absorption by the ocular media is small, therefore a considerable penetration of ultra-violet to the retina is expected. For the human eye the ultra-violet transmission is a function of age,<sup>2</sup> depending mainly upon the transparency of the lens;<sup>3</sup> it is maximal in infancy and thereafter decreases so that in the adult eye

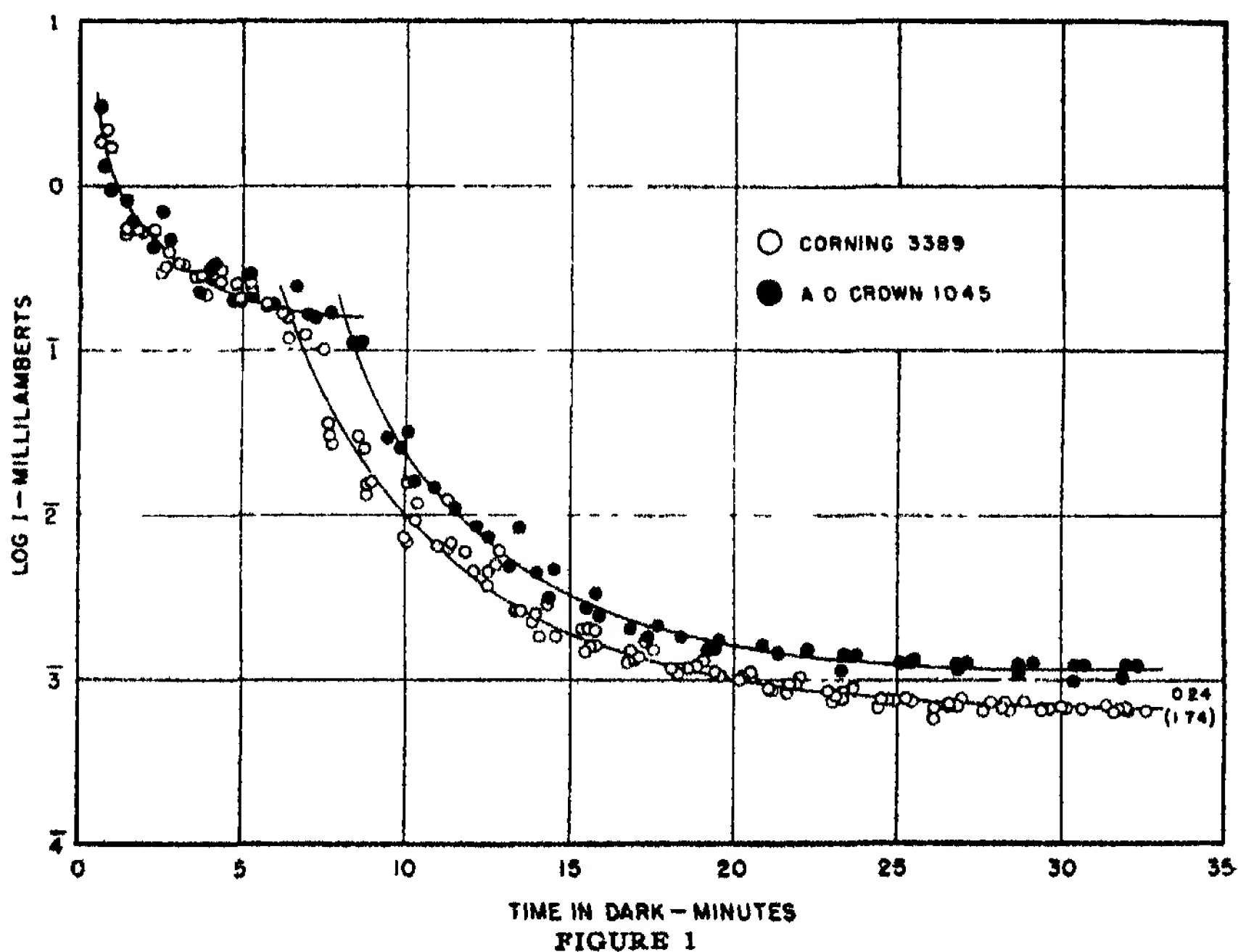
the lens transmission at  $365\text{ m}\mu$  is only 0.1 per cent.<sup>4</sup> In accordance with this fact one might assume *a priori* that wave-lengths shorter than  $365\text{ m}\mu$  can have practically no effect upon the retina. In subsequent tests it is demonstrated that addition of ultra-violet to the adapting light during preexposure changes the course of human dark adaptation and raises the final level of adaptation, and thus decreases the sensitivity of the eye.

*Light Exposure.*—The observer must be fully light adapted before the course of dark adaptation can be studied. As light source, a 250-watt mercury vapor lamp (GE type H-5) is used. The lamp is mounted in a housing 50 cm. behind a finely ground round Pyrex plate (transmitting from  $285\text{ m}\mu$  up), 30 cm. in diameter, which acts as a diffusing screen. The observer views this evenly illuminated screen from a distance of 50 cm., securing uniform light exposure of a large retinal area. Between lamp and screen, filters of different transmissions can be inserted which provide for addition of smaller or larger portions of the ultra-violet part of the spectrum to the otherwise white exposure light. The filters used are: AO crown 1045, transmitting from  $290\text{ m}\mu$  up; ordinary plate glass, transmitting above  $315\text{ m}\mu$ ; AO Cruxite 1794 transmitting above  $355\text{ m}\mu$ ; and Corning 3389 (Noviol, shade A), which begins to transmit at about  $410\text{ m}\mu$ . All filters have practically the same transmission in the visible, therefore the color of the exposure light does not change. The brightness of the screen as measured with a Macbeth Illuminometer is 6250 millilamberts. To this illumination varying only in ultra-violet content the observer is exposed prior to test for a standard period of 10 minutes.

*Measurements.*—The course of dark adaptation is followed with the aid of a visual discriminometer,<sup>5</sup> an instrument of highest experimental rigidity, particularly in regard to fixation, retinal location of the test field, size of the field and control of threshold intensities of light. The instrument is calibrated by insertion of the illuminometer into one tube of the binocular head, after removal of the eyepiece. After completion of light exposure the observer views monocularly a red fixation point, representing the center of a square test field, subtending a visual angle of  $12.5^\circ$  on a side. With good fixation and the head securely on a chin rest, the test field is presented by means of a shutter for  $1/25$  second, while by a neutral-tint wedge the operator of the instrument increases step by step the intensity of the field for each flash, until the critical point of first perception of the flash is reached. The time from cessation of light exposure and the wedge reading is recorded. This procedure is continued for 30 to 35 minutes, until there is no further increase in sensitivity at complete adaptation.

With exposure light free from ultra-violet (Noviol A in the light path) the ensuing dark adaptation curve is duplex, separating the process of adaptation into a cone and a rod component. The cone segment of the curve covers about 1.3 log units between the first reading, taken roughly at 1

minute, and the onset of the rod segment of the curve. The transition from cone to rod adaptation occurs after about 6.5 minutes. By adding to the exposure light ultra-violet down to  $290\text{ m}\mu$  by the insertion of crown glass into the light path instead of Noviol, the course of adaptation is modified. While the general level of the cone curve remains unchanged, it is extended up to 8 minutes, overshooting the previous onset of rod adaptation by  $1\frac{1}{2}$  minutes. Rod adaptation then follows a course parallel to that obtained without the ultra-violet but on a higher intensity level, so



The course of dark adaptation of the human eye after preexposure to light free from ultra-violet (Corning 3389, Noviol, shade A) in the light path (open circles) and light containing ultra-violet as low as  $290\text{ m}\mu$  (Crown in the light path), black circles. The ultra-violet produces a later onset of rod adaptation and raises the threshold levels for the rod segment.

that at complete adaptation the two curves are separated by 0.24 log unit which corresponds to an increase in threshold intensity by a factor of 1.74 for threshold recognition.

In figure 1 summarized data on one observer are presented. There are plotted 6 runs with Noviol and 3 runs with crown in the path of the exposure light. The observations extend over several days. A run with crown may follow a run with Noviol immediately. After crown a minimum time of 5 hours was allowed so that no previous ultra-violet effects would inter-



fere with a subsequent run. The data in figure 1 represent the prototype of the course of dark adaptation without and under the influence of ultra-violet in the exposure light. Altogether, measurements were taken on 6 observers which show in principle the same kind of phenomena. In each case a higher "final" threshold of the rod segment is found after pre-exposure to light filtered by crown only. The rise in "final" threshold varies between 0.21 and 0.29 log unit. The variation does not seem correlated to age, at least within the age range of the experimental group, nor to sex. In no case could any significant change in the level of the cone segment be observed. The overshooting of the cone part might vary from hardly detectable to 2 minutes. Also, the separation between the two curves over the steep part of the rod segment up to 20 minutes might be not as distinct as in figure 1. At 20 minutes and beyond the separation is, however, always clear and becomes increasingly greater, until the final level is reached. The final separation between the normal level of adaptation and the lowest level reached at termination of the experiment after exposure to ultra-violet, for all observers, is given below.

OBSERVER	AGE	SEX	MEAN SEPARATION IN LOG UNITS
T. H. C.	18	m	0.24
M. L.	19	f	0.24
E. E. F.	21	f	0.28
D. A. J.	25	f	0.24
G. A. B.	26	m	0.29
E. W.	43	m	0.23
Mean =			0.253

The mean increase in light intensity for threshold recognition among 6 observers is 0.253 log unit. This indicates that roughly 1.8 times as much light is needed for threshold response at "complete" dark adaptation after preexposure to light containing wave-lengths as low as 290  $m\mu$ .

Similar effects on final dark adaptation thresholds after prolonged exposure to sunlight, moderately rich in ultra-violet, have been described recently.<sup>6</sup> An overshooting of the cone segment and a temporary rise of final thresholds of the same order of magnitude as described here are noticed. Ultra-violet as a cause is not mentioned. It seems probable, however, according to some of our tests with preexposure to light reflected from snow, that the changes in final level are not due to glare, but rather to the ultra-violet.

On two observers tests were made comparing the effects of light filtered by Noviol with those of light filtered by plate glass, which excludes the strong mercury lines at 297, 302 and 313  $m\mu$ , but transmits the 365  $m\mu$  band at full strength. The data are given in figure 2. Besides a difference in the onset of rod adaptation for the two observers, both show an overshooting of the cone segment for the plate glass curves of about 1

minute. The mean final threshold difference is 0.15 log unit, 0.1 log unit less than with crown glass, due to the reduction in effective ultra-violet radiation.

Comparing AO Cruxite which transmits about 20 per cent at 365  $m\mu$  with Noviol, the ensuing dark adaptation curves are practically identical. At least no final threshold differences can be noticed. There are, however, slight irregularities at the cone-rod transition which suggest overshooting

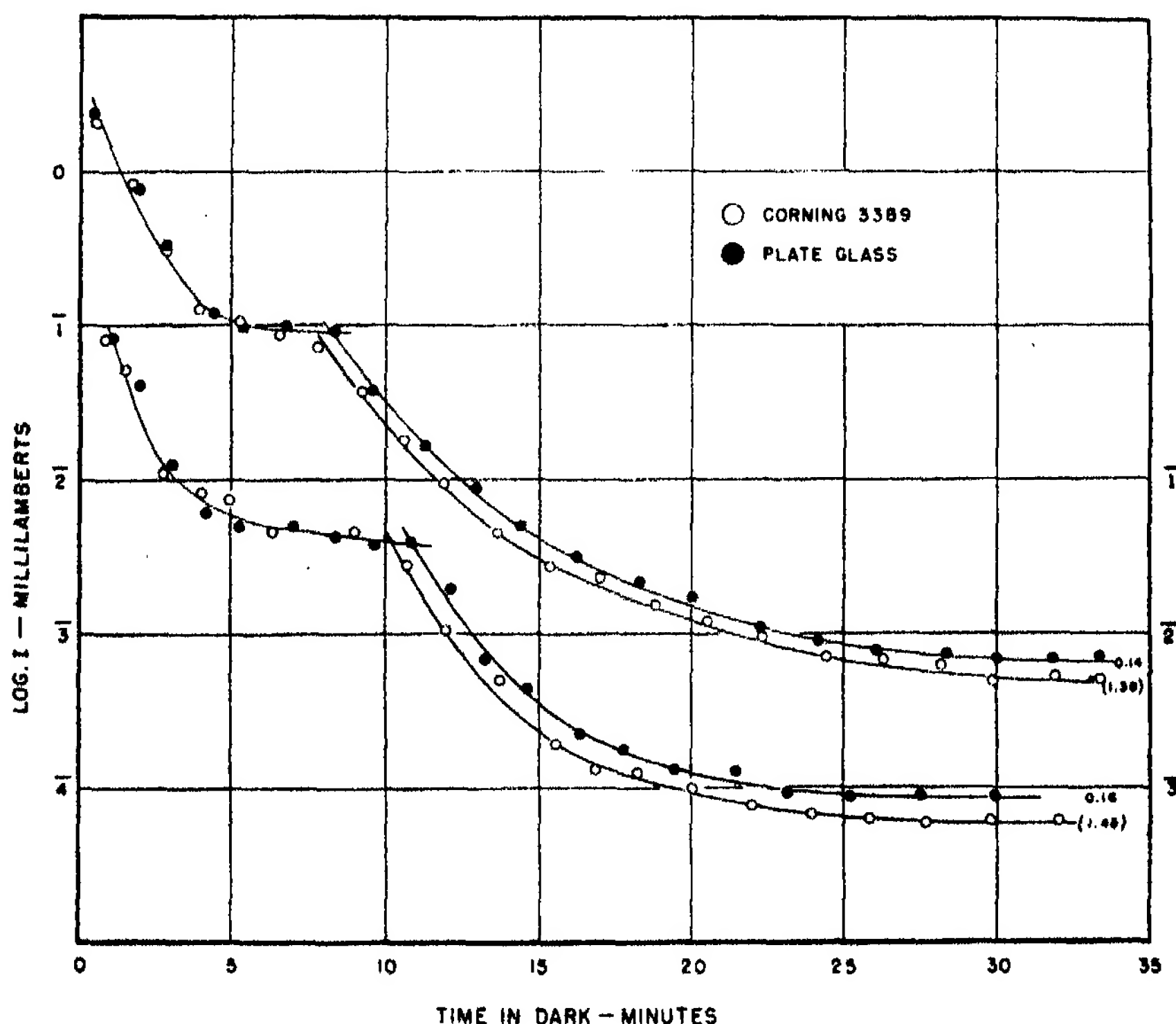


FIGURE 2

The course of dark adaptation after preexposure to light free of ultra-violet (open circles) and light filtered by plate glass (black circles). Due to the ultra-violet, the rod adaptation begins later and the thresholds are higher.

of the Cruxite curve. Also the initial steepness of the rod segment might be less for Cruxite. From 15 minutes onward the normal and the Cruxite curves are, however, identical, indicating that any small ultra-violet effect produced by the reduced transmission at 365  $m\mu$ , recognizable at the beginning of rod adaptation, has disappeared at a time when previously the effect of exposure to ultra-violet was most pronounced.

Compared with the results on the eyes of baby chicks the effect on human dark adaptation is considerably smaller. In the chick an intensity increase by a factor of 12.5 was necessary (crown vs. Noviol) as against 1.8 for the human eye, a ratio of 6.6:1. Assuming that most of the effective ultra-violet is absorbed by the lens, one should expect a considerably

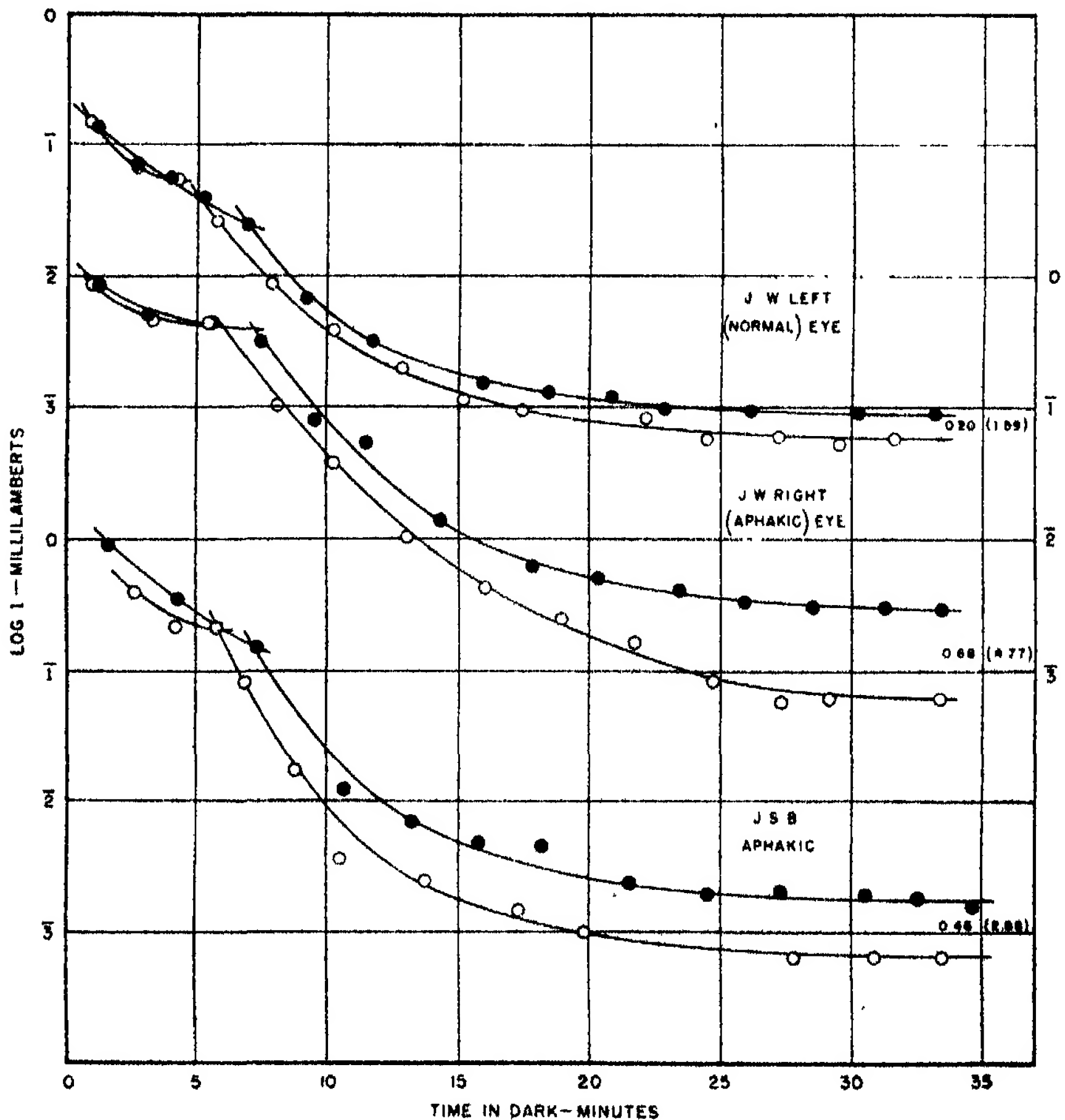


FIGURE 3

Dark adaptation in aphakics: The normal (left) eye of J. W. gives curves similar to those presented in figure 1. For the aphakic eye the separation between the normal and the ultra-violet curve is considerably increased, due to more effective action of ultra-violet upon the retina. The same conditions are found in J. S. B.

greater effect in aphakics. Two observers were available. J. W., age 26, had the lens of his right eye removed several years ago, the left eye having normal vision. His normal eye gives a final threshold separation of 0.20

log unit which is slightly below the average of the group of 6 mentioned previously. For the aphakic eye the separation between the Noviol and the crown curves becomes increasingly greater after the cone-rod transition, until a final separation of 0.68 log unit is reached, corresponding to an increase in light intensity by a factor of 4.77 for threshold recognition. In J. S. B., 30 years, both eyes are aphakic; one eye having better vision is used for test. The results are similar to those for J. W. with the exception that the final separation is only 0.46 log unit, or a factor of 2.88. Even while there is a difference of 0.22 log unit between the two aphakics, the final threshold levels for both are so much higher than previously found that it becomes evident that much of the ultra-violet is normally absorbed by the lens which otherwise would reach the retina. The data on the aphakic observers are presented in figure 3.

With only 0.1 per cent transmission at  $365\text{ m}\mu$ , there is no doubt that the small amount of ultra-violet reaching the retina produces considerable physiological effects in proportion to its intensity. In the human eye, as well as in the chick, the ultra-violet effect seems entirely on the rod thresholds, whereas the cones remain unaffected. For both types of eyes it may be assumed that an effect on the cones is prevented by their dense filter pigments, while the ultra-violet can act upon the pigment-free rods.<sup>7</sup> The action of the ultra-violet might be directly upon the photosensitive material of the rods, or it might be due to desensitization caused by fluorescence of the ocular media during preexposure. In both cases a longer recovery time would be needed to regain maximal sensitivity. At present it can only be pointed out that ultra-violet radiation has peculiar effects upon the sensitivity thresholds of the rods, disregarding the locus and mode of action.

*Summary.*—The course of dark adaptation of the human eye is studied after preexposure to the radiation of a mercury vapor lamp, filtering out the ultra-violet between  $290\text{ m}\mu$  and the visible to various extents. Exposure to light free from ultra-violet results in uniform dark adaptation curves. Addition of ultra-violet below  $365\text{ m}\mu$  affects rod adaptation by causing a later onset of rod adaptation and raising the final thresholds appreciably above the normal level. The cone adaptation is not affected. The final level reached is a function of the extent of the ultra-violet spectrum. The lens, having a high ultra-violet absorption, reduces the ultra-violet action upon the retina which can be demonstrated on aphakic eyes in which the ultra-violet action on dark adaptation is considerably increased.

\* This research was supported by a grant of the American Optical Company.

† The experiments were carried out with a visual discriminometer at the Laboratory of Industrial Physiology, Harvard School of Business Administration, which kindly was

made available for the purpose. I feel particularly indebted to Miss D. A. Jameson for her generous help in these experiments.

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*EXPERIMENTS ON SEXUAL ISOLATION IN DROSOPHILA.*  
*VIII. INFLUENCE OF LIGHT ON THE MATING BEHAVIOR OF*  
*DROSOPHILA SUBOBSCURA, DROSOPHILA PERSIMILIS AND*  
*DROSOPHILA PSEUDOOBSCURA*

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Philip, Rendel, Spurway and Haldane<sup>1</sup> and Rendel<sup>2</sup> found that cultures of *Drosophila subobscura* Collin cannot be kept permanently in a dark room because no matings take place in this species in the absence of light. Furthermore, several mutant types with abnormal eye colors proved to be male sterile; for example, white eyed males, although they are positively phototropic like the wild type, do not respond to moving contours and produce no offspring. Rendel<sup>2</sup> also points out that the males of the mutant yellow (yellow body color) are discriminated against in mating by the females of some, but not all, wild type strains. Yellow females produce offspring easily when placed with wild type males. These findings are the more interesting and unexpected since Mayr and Dobzhansky<sup>3</sup> found that in *D. persimilis* Dobzhansky and Epling and in *D. pseudoobscura* Frolova mating and insemination occur freely in the presence or absence of light. Cultures of these species can be kept in a dark room generation after generation. Furthermore, when females of these two species are placed together with males of one of them, a significantly greater proportion of conspecific than of foreign females are inseminated and this selectivity of mating is not affected by light or by darkness. It follows that representatives of these species are able to discriminate between conspecific and foreign mates in the absence of light.



*D. subobscura*, *D. persimilis* and *D. pseudoobscura* are three morphologically very similar species; yet their mating reactions are profoundly different with respect to at least one environmental agent, namely, light. Such variations in the mating reactions in related species may be very important biologically if they lead to sexual isolation between the species. It might be interesting to resolve some representative examples of sexual isolation into their component parts and to identify the variables which enter into a sexual isolation. Such differences in the behavior of related species as the dependence or independence of mating on presence or absence of light lend themselves readily to quantitative and qualitative study. The results of an exploratory study of this type is reported in the present paper.

We wish to express our sincere appreciation of the courtesy of Professor J. B. S. Haldane who let us have a strain of *D. subobscura* collected in England and bred in his laboratory. Professor H. J. Muller very kindly transported this strain to this country. Professor S. Hecht and Drs. S. Shlaer and Ch. Hendley of the Laboratory of Biophysics, Columbia University, have very kindly helped to arrange the experiments on light intensities. To Mr. George Streisinger we are obliged for his assistance with the experiments at the beginning of the investigation.

*Material and Methods.*—A single strain of each of the three species has been used in all the experiments to be described: the English strain of *D. subobscura*, *D. persimilis* from Stony Creek, California, and the orange mutant strain of *D. pseudoobscura*. Adults of *D. subobscura* were transferred without etherization to fresh culture bottles at intervals of from one to several days and kept in a well-lighted laboratory room at 22°–24°C. The bottles with eggs deposited in them were placed in a dark constant temperature room at 19°C. Cultures of *D. persimilis* and *D. pseudoobscura* were treated similarly except that they were allowed to develop at room temperature.

*Effects of Light and Darkness on the Frequency of Insemination.*—The experiments consisted of placing 10 to 15 females of one species with a similar number of males of the same or of a different species in 1 × 3¼-inch glass vials with food and examining the females for the presence of sperm after a certain number of days. At the beginning of each experiment the flies were not more than 24 hours old after hatching from the pupae. Some of the vials (light series) were placed on a shelf in the laboratory out of the direct sunlight but exposed to the diffuse light of the room. Other vials (dark series) were individually wrapped in heavy black paper and placed in an opaque cardboard box which was then put on the shelf with the vials of the light series. After a desired number of days, all females were dissected and their seminal receptacles were examined under a microscope for sperm.<sup>1</sup> The data obtained in the parallel experiments of the light and dark series are summarized in table 1.

TABLE 1  
NUMBER OF FEMALES DISSECTED (n) AND PER CENT FERTILIZED (%) AFTER SEVERAL DAYS' EXPOSURE IN LIGHT AND IN DARKNESS

CROSS	LIGHT			DARK		
	DAYS	n	%	DAYS	n	%
subobscura ♀ × subobscura ♂	3-4	60	80.0	5-9	72	0.0
persimilis ♀ × persimilis ♂	3-5	41	75.6	9	22	100.0
subobscura ♀ × persimilis ♂	7-14	118	8.5	7-9	136	2.9
persimilis ♀ × subobscura ♂	7-13	80	15.0	7-12	80	1.3*
subobscura ♀ × pseudoobscura ♂	9-11	64	21.9	8-15	152	7.2
pseudoobscura ♀ × subobscura ♂	9-13	96	25.0	10-15	128	0.0

\* A single female found to contain sperm in this cross is probably due to an experimental error.

It can be seen that none of the 72 *D. subobscura* females were inseminated by males of their own species after as long as 5-9 days together in darkness. By contrast, about 80% of the females were inseminated after 3-4 days in the light. The behavior of *D. persimilis* is quite different since males of this species, as well as of *D. pseudoobscura*, inseminate females of their respective species equally as rapidly in light as in the dark (for more data on the latter species see Mayr and Dobzhansky<sup>8</sup>).

Hybrids of *D. persimilis* and *D. pseudoobscura* can be obtained in the laboratory rather easily. The viability of these F<sub>1</sub> hybrids is not inferior to that of the parents. Crosses of *D. subobscura* with *D. persimilis* or with *D. pseudoobscura* never produced viable hybrids, larvae or adults, in our experiments. Nevertheless, as shown in table 1, cross-insemination between these species does occur although much less frequently than matings within a species. In the light, *D. subobscura* males have inseminated as many as 25% of *D. pseudoobscura* and 15% of *D. persimilis* females in 7-13 days. No inseminations by *D. subobscura* males have been observed in the dark series. (One female of *D. persimilis*, among 80 females exposed for 7-12 days to *D. subobscura* males, contained sperm, cf. table 1, but this is almost certainly an experimental error.)

Although *D. subobscura* females and males do not mate in the dark, an appreciable number of inseminations have been observed in the crosses that involve *D. subobscura* females and *D. persimilis* or *D. pseudoobscura* males. These interspecific matings have been observed both in the light and in the dark series but, interestingly enough, they are relatively more frequent in the light than in the dark. Light is essential for normal sexual activity of *D. subobscura* males. *D. subobscura* females accept some courting males in the dark although they do so less frequently than in the light. Light is not an essential factor for mating of either males or females of *D. persimilis* or of *D. pseudoobscura*.

The following experiment was intended to clarify the role of the light in the mating behavior of *D. subobscura*. Several vials each containing 10

males and 10 females of *D. subobscura* were prepared. These vials were placed in a small opaque box which was, in turn, placed in a larger box. The vials and boxes were kept in a drawer of a desk. Several times each day for 8–10 days, these vials were exposed to the light of the room (several times to direct sunlight) until the first courtship was observed in any of the vials; the vials were then quickly shaken, covered and replaced in the desk. After 8 days, 15 females were dissected and after 10 days, 18 more. None of these females had been inseminated. Apparently darkness prevents the completion of matings which have proceeded to the courtship stage. There seems to be no appreciable carrying over of sexual activity from periods of light exposure into darkness.

In order to test the rapidity of matings of *D. pseudoobscura* in the dark, vials with food containing 10 males and 10 females were prepared and placed within the series of boxes mentioned above. Females were dissected at the end of 1, 2, 4 and 7 hours. All of the females were inseminated in those vials which were left for 2 hours or more; 7 females in the vial left for 1 hour contained sperm. Another series of vials was prepared without food and placed in the dark for times ranging from 15 to 90 minutes (15-minute intervals). The number of inseminated females varied from 8 after 30 and 45 minutes of exposure to 4 after 1 hour. Exposure for 1 1/2 hours and 15 minutes gave identical results—7 females inseminated. Activity in the dark, then, begins at once and proceeds at a pace comparable to that to be described in the next experiment.

*Direct Observations on the Sexual Behavior.*—Females and males of all three species were aged for 1–2 weeks in isolation in a dark room at 19°C. Clean vials without food were placed on vial racks covered with white paper; 2 females of one species and 2 males of the same or of a different species were introduced, without etherization, into each vial. The behavior of the flies in the vials were then observed for two hours under normal daylight conditions on a table before a window (direct sunlight being avoided). Ordinarily 20 vials were under observation at one time; 5 vials of each of the four possible combinations  $A\sigma \times A\varphi$ ,  $A\sigma \times B\varphi$ ,  $B\sigma \times A\varphi$ ,  $B\sigma \times B\varphi$ , where A is *D. subobscura* and B is *D. pseudoobscura* or *D. persimilis*. At times it was expedient to increase the number of vials of a certain cross at the expense of other crosses, but never more than 20 vials were used simultaneously since this was found to be the maximum number that could be handled efficiently.

Three stages can be distinguished in the mating of *Drosophila*: courtship, copulation and insemination. The occurrence or non-occurrence of the last of these stages, insemination, can be determined with certainty by dissection of the female and microscopic examination of her sperm receptacles and vagina. It is slightly more difficult to decide whether copulation has or has not taken place. As a rule, absence of space between the tips of the

abdomens of the flies involved and difficulty of the female in dislodging the male were taken as evidence that copulation had occurred. The occurrence of courtship is the most difficult to ascertain and much depends upon an individual observer. Detailed descriptions of the courtships of *D. subobscura* has been given by Rendel<sup>2</sup> and of *D. persimilis* and *D. pseudoobscura* by Mayr.<sup>4</sup> These descriptions have been confirmed by us. The courtships involve males sparring with females, wing flicking, wing extension and vibration, and finally circling and mounting. All three species indulge in sparring, i.e., exchanging of taps with the forelegs. *D. subobscura* males flick their wings while males of the other two species do not. Wing extension in *D. persimilis* and *D. pseudoobscura* consists of extending one wing parallel to the female's body, usually anteriorly, while the male stands facing the female from either side. In *D. subobscura* wing extension occurs while the male stands in front of and facing the female and consists of extending both wings at right angles to the male's body.

Males often court other males but such courtships were not included in the data. Since courtships are not always continued after sparring and since sparring is of short duration and liable to be overlooked, sparring alone was not counted as courtship. *D. subobscura* males often indulge in wing flicking on occasions other than courtships, hence flicking was not considered to constitute a courtship unless the male did it insistently before a female. Extending and vibrating a wing(s) was taken in all cases to be a bona fide courtship. If a courting pair separated and there was no attempt on the part of the male to continue the courtship, that courtship was regarded completed. If, after a lapse of time, the same male resumed courting, this fact was recorded as a separate courtship. However, if the male followed a female and his behavior seemed to indicate a continuing interest in her, his entire performance was considered a single courtship. The total results of the two-hour observational periods are given in table 2. Table 3 shows some of the data reported in table 2 recalculated for a single female for the four consecutive half-hour periods of observation.

TABLE 2  
NUMBER OF COURTSHIPS, COPULATIONS AND INSEMINATIONS DURING TWO-HOUR  
EXPOSURE PERIODS OF AGED FLIES UNDER NORMAL DAYLIGHT CONDITIONS

CROSS	FEMALES EXPOSED		COURTSHIPS		COPULATIONS		INSEMINATIONS	
		TOTAL	PER ♀		TOTAL	PER ♀	TOTAL	PER ♀
<i>subobscura</i> ♀ × <i>subobscura</i> ♂	86	118	1.37		17	0.20	..	..
<i>persimilis</i> ♀ × <i>persimilis</i> ♂	60	61	1.02		32	0.53	..	..
<i>pseudoobscura</i> ♀ × <i>pseudoobscura</i> ♂	66	104	1.58		40	0.61	..	..
<i>subobscura</i> ♀ × <i>persimilis</i> ♂	60	52	0.87		0	0	0	0
<i>persimilis</i> ♀ × <i>subobscura</i> ♂	60	186	3.10		50	0.83	8	0.10
<i>subobscura</i> ♀ × <i>pseudoobscura</i> ♂	70	81	1.16		0	0	0	0
<i>pseudoobscura</i> ♀ × <i>subobscura</i> ♂	68	88	1.29		5	0.07	0	0



TABLE 3

COURTSHIPS (CRT) AND COPULATIONS (COP) PER FEMALE BY HALF-HOUR INTERVALS OF EXPOSURE

CROSS		1ST	2ND	3RD	4TH	TOTAL	FEMALES EXPOSED
subobscura ♀ × subobscura ♂	{Crt	0.45	0.23	0.13	0.18	0.98\	56
	{Cop	0.07	0.02	0.02	0.02	0.13\	
persimilis ♀ × persimilis ♂	{Crt	0.67	0.37	0.10	0.03	1.17\	30
	{Cop	0.53	0.30	0.07	0	0.90\	
pseudoobscura ♀ × pseudoobscura ♂	{Crt	0.59	0.47	0.32	0.20	1.58\	66
	{Cop	0.30	0.14	0.11	0.06	0.61\	
subobscura ♀ × persimilis ♂	{Crt	0.17	0.13	0.30	0.07	0.67\	30
	{Cop	0	0	0	0	0\	
persimilis ♀ × subobscura ♂	{Crt	1.73	0.73	0.60	0.33	3.40\	30
	{Cop	0.60	0.20	0.07	0.07	0.93\	
subobscura ♀ × pseudoobscura ♂	{Crt	0.27	0.26	0.34	0.29	1.16\	70
	{Cop	0	0	0	0	0\	
pseudoobscura ♀ × subobscura ♂	{Crt	0.37	0.40	0.24	0.29	1.29\	68
	{Cop	0.03	0.04	0	0	0.07\	

An inspection of table 2 reveals facts that could not have been predicted on the basis of the data presented in table 1, which shows interspecific inseminations to be much less frequent than intraspecific ones. Table 2 shows that the number of courtships per female is of the same order of magnitude in all crosses. In fact, the greatest number of courtships per female occur when *D. subobscura* males are placed with *D. persimilis* females and the smallest number in the reciprocal cross. Table 3 indicates, however, that this uniformity is arrived at differently in the interspecific and intraspecific crosses. When the females and males belong to the same species, the number of courtships declines rapidly in the consecutive half-hour periods. When the females and males belong to different species, the frequency of courtships remains more nearly constant throughout the two hour period. The most probable explanation of these relationships lies in that a much higher proportion of intraspecific than of interspecific courtships result in copulations. (See below.) Consequently, the courtship behavior subsides more rapidly in the vials containing conspecific females and males than in vials containing representatives of different species.

During the first half hour of observation, when few of the individuals had copulated previously, a high proportion of the courtships between males and females of the same species are followed by copulations. Data in table 3 show that the ratio copulations/courtships is highest in *D. persimilis*, intermediate in *D. pseudoobscura* and lowest in *D. subobscura*. Behavior within the three species is similar: genitalia are brought into contact, the male mounts and the pair remains quiet from 3 to 10 minutes (average 6½ minutes) after which separation occurs. Whenever dissected the females



contained spermatozoa. In the combinations of *D. subobscura* females with *D. persimilis* or *D. pseudoobscura* males (tables 2 and 3), no normal copulations at all have been observed although they must take place as inseminations have been recorded for these crosses (table 1). The female runs away from the courting male, repulses him by extending her middle leg toward him, rotates her abdomen, or else the male breaks off the courtship without attempting to mount. If he mounts, as he frequently does, the pair separate immediately. The situation, therefore, is analogous to that found by Mayr<sup>4</sup> in the crosses of *D. persimilis* and *D. pseudoobscura*. The precise nature of the difficulty which prevents the copulation from being successful is not clear. It may be noted that the external genitalia are morphologically identical in *D. persimilis* and *D. pseudoobscura*, while those of *D. subobscura* males differ in several characters both of the genital arch, the anal tubercle and the penis apparatus.

When *D. subobscura* males and *D. pseudoobscura* or *D. persimilis* females, especially the latter, are involved, things go differently. The male mounts and copulation seems to be successful but in a few seconds the female begins to struggle in violent efforts to free herself from the male. The pair frequently falls on the bottom of the vial, the male attempting to maintain his position and the female striving to dislodge him with kicks by her hind legs. The female succeeds in freeing herself in from a few seconds to about two minutes after the start of the struggle; less than 30 seconds is required in the majority of cases.

Only 6 out of the 50 *D. persimilis* females and none of the *D. pseudoobscura* females which have copulated with *D. subobscura* males contained any spermatozoa on dissection. This means that a majority of the interspecific copulations do not result in sperm delivery. It can be surmised that insemination occurs only in those cases when copulation lasts longer than the average.

*Intensity of Illumination and Sexual Activity of D. subobscura.*—The courtships and copulations of *D. subobscura* males and females were observed by placing vials each containing 2 females and 2 males at various distances (1–5 meters) from a 150-watt Reflectorflood bulb in a dark room at the Department of Biophysics of Columbia University. Observations were made by walking from vial rack to vial rack. Each experiment lasted for two hours. Ordinarily two vials were placed at each position. Inasmuch as a courtship is of such short duration that it can begin and end in the time it takes an observer to make a circuit of all vials ( $\approx 1$  min.) while a copulation lasts for several minutes, the ratios copulations/courtships in the present data are not comparable to those obtained from the above data. Table 4 summarizes the results of these observations.

TABLE 4  
COURTSHIPS AND COPULATIONS IN *D. subobscura* AT DIFFERENT LIGHT INTENSITIES  
(FOOT-CANDLES)

LIGHT INTENSITY	TOTAL FEMALES	COURTSHIPS		COPULATIONS	
		TOTAL	PER ♀	TOTAL	PER ♀
85.4	22	8	0.36	2	0.09
27.0	22	59	2.68	8	0.36
10.3	22	29	1.32	1	0.05
4.5	22	7	0.32	2	0.09
2.5	20	14	0.70	1	0.05

Table 4 shows that both very bright and dim light are unfavorable for sexual activity. The flies were most active when placed in light of 27 foot-candle intensity. Sexual activity seems to be correlated in this case with mobility of the flies because in the vials in which many courtships were taking place, the flies were moving about to a greater extent than in the others. The latter point is borne out in that flies placed behind a black screen in the same dark room and observed by light reflected from a white card were very quiet, moved very slowly if at all, and never sparred, courted or copulated. It must be admitted, however, that it is difficult to observe the behavior of flies accurately in light of less than 2.5 foot-candles intensity.

*Behavior of D. subobscura in Red Light.*—Bertholf<sup>5</sup> has shown that wild type *D. melanogaster* flies are relatively insensitive to red light and that the upper limit of sensitivity lies at 650–675  $\mu$  wave-length. Observations on the behavior of the flies in red light, therefore, should give an approximate idea about their behavior in the absence of light. A glass filter transmitting light of 600–610  $\mu$  was placed in the lamp used in the previous experiments. Energy and intensity measurements were not made; intensity of red light in foot-candles is meaningless and energy is unimportant inasmuch as a point of non-activity rather than a threshold was what was desired.

When the two vials each containing two pairs of *D. subobscura* were placed at the distances from the light source mentioned above, no sexual activity of any type was observed. The flies remained quiet or moved slowly in the vial. There was no orientation of the flies with respect to the light.

When the flies used were *D. pseudoobscura*, courtships and copulations proceeded as they do in the light. Although, in general, there was no change in behavior, male flies were observed on two occasions extending and vibrating their wings towards the posterior end of the females and attempting to mount the anterior end.

Since the absence of activity in *D. subobscura* and the presence of activity in *D. pseudoobscura* in red light resemble the situation existing in the absence of light, vials containing five females of one species and five males of the same or the other species were prepared and observed for two hours.

There was no indication of courtships on the part of *D. subobscura* males either with their own or with *D. pseudoobscura* females. *D. pseudoobscura* males courted and copulated with their own females and courted *D. subobscura* females. As in the observations recorded in table 2, no copulations of *D. pseudoobscura* males with *D. subobscura* females were seen; each courtship was terminated when the female moved away or rotated her abdomen.

*Summary.*—Mating takes place with or without light in *Drosophila persimilis* and *D. pseudoobscura*, while *D. subobscura* mates only in the presence of light. Males of *D. persimilis* and *D. pseudoobscura* inseminate some *D. subobscura* females, the frequency of this cross-insemination being greater in the light than in the dark. *D. subobscura* males inseminate some females of the other two species in the light but not in the dark. Light intensity of the order of 30 foot-candles was found to be close to the optimum for mating in *D. subobscura*, but no mating and no courtship take place in this species in red light of an intensity which permits observation. Direct observations disclose that, in the light, males of any one of the three species court about equally frequently females of their own and of the other two species. However, interspecific copulation occurs only seldom, and if it does the female dislodges the male in usually less than 30 seconds, ordinarily before sperm ejaculation takes place.

<sup>1</sup> Philip, U., Rendel, J. M., Spurway, H., and Haldane, J. B. S., *Nature*, 154, 260-262 (1944).

<sup>2</sup> Rendel, J. M., *Jour. Genetics*, 46, 287-302 (1945).

<sup>3</sup> Mayr, E., and Dobzhansky, Th., these PROCEEDINGS, 31, 75-82 (1945).

<sup>4</sup> Mayr, E., *Ibid.*, 32, 128-137 (1946).

<sup>5</sup> Bertholf, L. M., *Zeits. vergl. Physiol.*, 18, 32-64 (1932).

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## NOTE ON THE LOCATION OF THE ZEROS OF THE DERIVATIVE OF A RATIONAL FUNCTION HAVING PRESCRIBED SYMMETRY

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The object of this note is to state without detailed proof some new results, representing a sharpening of some known results on the subject of the title.

Jensen's theorem is classical: *If  $f(z)$  is a real polynomial, then all non-real zeros of the derivative  $f'(z)$  lie on or within the circles (Jensen circles) constructed with diameters the segments joining pairs of conjugate imaginary zeros of  $f(z)$ .*

For any fixed polynomial  $f(z)$  which has non-real zeros, the point set assigned to zeros of  $f'(z)$  by Jensen's theorem is unnecessarily large, and can be made smaller by use of Lucas' theorem, that if a convex region  $K$  contains the zeros of a polynomial  $f(z)$ , then  $K$  also contains the zeros of the derivative  $f'(z)$ . In one special case the theorem of Jensen can be considerably improved:

**THEOREM 1.** *Let  $f(z)$  be a real polynomial with precisely one pair  $(\alpha, \bar{\alpha})$  of conjugate imaginary zeros. Let  $z_0$  be the algebraically least and  $z_1$  the algebraically greatest of the real zeros of  $f(z)$ . Let  $A_k$  ( $k = 0, 1$ ) be the circular arc bounded by  $\alpha$  and  $\bar{\alpha}$  of angular measure less than  $\pi$  and tangent to the lines  $\alpha z_k$  and  $\bar{\alpha} z_k$ . Then all non-real zeros of  $f'(z)$  lie in the closed lens-shaped region bounded by  $A_0$  and  $A_1$ .*

Theorem 1 admits of extension to the case where  $f(z)$  has more than one pair of non-real zeros. Here to modify one Jensen circle the rôles of  $z_0$  and  $z_1$  of Theorem 1 may be taken by real zeros of  $f(z)$  or by points easily constructed from other Jensen circles. The extended theorem assigns a point set for possible positions of zeros of  $f'(z)$  which cannot be improved by use of Lucas' theorem.

Jensen's theorem (like Lucas' theorem) admits extension to the non-Euclidean (hyperbolic) plane:

**THEOREM 2.** *Let  $f(z)$  be a real rational function which has no poles interior*

to the unit circle  $C$  and whose zeros are inverse to its poles with respect to  $C$ . Denote by Jensen circles the non-Euclidean circles constructed using non-Euclidean segments joining pairs of conjugate imaginary zeros of  $f(z)$  as non-Euclidean diameters. Then all non-real zeros of  $f'(z)$  interior to  $C$  lie on or within these Jensen circles.

Theorem 2 may be proved by considering in the extended plane the field of force due to suitable repelling and attracting particles situated at the zeros and poles of  $f(z)$ ; all zeros of  $f'(z)$  not zeros of  $f(z)$  are positions of equilibrium in this field of force. At an arbitrary point  $z$  interior to  $C$  but not on  $Ox$  nor on or within a Jensen circle, the force has a non-zero component away from  $Ox$  in the direction of the non-Euclidean line through  $z$  perpendicular to  $Ox$ , and this is true for the total resultant force, or for the force due to a pair of particles on  $Ox$  situated in a corresponding zero and pole of  $f(z)$ , or for the force due to four particles situated in a pair of conjugate imaginary zeros of  $f(z)$  and their corresponding poles.

Jensen's theorem is a limiting case of and can be proved from Theorem 2.

It may be noted that Theorem 2, generalized by a conformal map, can be expressed as follows:

*Let  $R$  be a simply connected region which is symmetric in  $Ox$ , and let the function  $f(z)$  be real on  $Ox$ , analytic interior to  $R$ , continuous in the corresponding closed region, and of modulus unity on the boundary of  $R$ . Then all non-real zeros of  $f'(z)$  lie on or within the non-Euclidean circles constructed on the pairs of non-real zeros of  $f(z)$  as non-Euclidean diametrically opposite points.*

A different type of symmetry leads to different conclusions:

**THEOREM 3.** *Let  $f(z)$  be a rational function whose poles are symmetric to its zeros in the origin  $O$ . Let the halves of a double sector with vertex  $O$  contain, respectively, all the zeros of  $f(z)$  and all the poles of  $f(z)$ . Then all the zeros of  $f'(z)$  also lie in this double sector. If the angle of the double sector is not greater than  $\pi/2$ , then any circle whose center is  $O$  containing all [or no] zeros of  $f(z)$  contains all [or no] zeros of  $f'(z)$ .*

If the angle of the sector here is greater than  $\pi/2$ , a new circle can be assigned containing all [or no] zeros of  $f'(z)$ .

There are two categories of results concerning the location of zeros of the derivative of a rational function: (a) those which do not depend on the relative multiplicities of the zeros (although symmetry may be prescribed) and (b) those which do depend on the multiplicities. Illustrations of (a) are Theorems 1, 2 and 3. In the study of the location of the zeros of the derivative  $f'(z)$  of a rational function  $f(z)$ , we ordinarily interpret the zeros of  $f'(z)$  which are not also zeros of  $f(z)$  as the positions of equilibrium in the field of force due to suitable positive particles situated at the zeros of  $f(z)$  and negative particles at the poles of  $f(z)$ . Whenever situations of type (a) are studied, it is useful to separate the zeros and poles of



the given  $f(z)$  into groups, chosen as small as possible yet retaining the prescribed symmetry and serving as components in the construction of  $f(z)$ . For instance in Jensen's theorem a group of zeros of  $f(z)$  consists either of a pair of conjugate imaginary zeros or of a single real zero; all poles of  $f(z)$  are at infinity. In the general case, for each pair of groups we define the  $W$ -curve, namely, the locus of all positions of equilibrium in the field of force due to the two groups of particles where the particles are considered fixed in position with arbitrary (i.e., not necessarily integral) variable multiplicities; but in varying the multiplicities it is not intended that a pole of  $f(z)$  should be allowed to replace a zero or reciprocally. Then for the given positions of zeros and poles but with arbitrary multiplicities, *the locus of the zeros of  $f'(z)$  is bounded by the  $W$ -curves taken for all possible pairs of groups of zeros and poles of  $f(z)$ .*

In the simplest cases, such as that of Lucas, or the case of a rational function whose zeros are symmetric to its poles with respect to a circle, or the case of a polynomial whose zeros occur in pairs symmetric with respect to a point—in these cases the  $W$ -curves and lines of force coincide. In more involved cases such as those of Jensen's theorem or the new results of the present note, the set of lines of force is different from the set of  $W$ -curves, and this fact makes proofs more complicated. Nevertheless the  $W$ -curves yield important information concerning the zeros of  $f'(z)$ .

Theorems 1, 2 and 3 can be applied to the study of the location of the critical points of harmonic functions under corresponding conditions of symmetry.

The writer plans to publish elsewhere detailed proofs of the theorems stated and others, with references to the literature.

## ON A THEOREM OF GELFAND AND NEUMARK

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Communicated July 26, 1946

Our purpose is to discuss the proof of the following theorem of Gelfand and Neumark:<sup>1</sup>

Let  $A$  be a complex Banach space which is at the same time a commutative ring with unit, multiplication satisfying

$$\|fg\| \leq \|f\| \|g\|,$$

and in which there is defined an automorphism (\*) such that

$$\|ff^*\| = \|f\| \|f^*\|, \quad (1)$$

$$\begin{aligned}(fg)^* &= f^*g^*, \\ (\lambda f + g)^* &= \bar{\lambda}f^* + g^*, \\ f^{**} &= f.\end{aligned}$$

Then there exists a compact Hausdorff space  $\Omega$  such that  $A$  is the ring of all continuous complex-valued functions on  $\Omega$ , and

$$\begin{aligned}\|f\| &= \sup_{x \in \Omega} |f(x)|, \\ f^*(x) &= \overline{f(x)}.\end{aligned}\tag{2}$$

The proof of Gelfand-Neumark uses the simple and elegant technique of normed rings due to Gelfand and others, except in one instance: the hypothesis (1) is used to establish (2) as a result of the existence of a unique minimal closed set  $\Omega_0 \subset \Omega$  having the property

$$\|f\| = \sup_{x \in \Omega_0} |f(x)|, \text{ for each } f \in A.$$

The authors refer to a rather inaccessible publication for the proof of the existence of this set  $\Omega_0$ .

■ We shall give here another way of deducing (2). In order not to repeat obvious portions of reference 1 which do not involve (1) we shall suppose that

$$|f(x)| \leq \|f\|, \text{ for } f \in A \text{ and } x \in \Omega,$$

has been already established,  $\Omega$  being the space of maximal ideals of  $A$ .

Now suppose  $f \in A$  and  $x \in \Omega$ , and

$$\begin{aligned}f(x) &= a + bi, \\ f^*(x) &= c + di.\end{aligned}$$

If  $b + d \neq 0$ , define

$$g = (f + f^* - au - cu)/(b + d),$$

where  $u$  is used to denote the unit element in  $A$ .

Then  $g = g^*$  and  $g(x) = i$ . This means  $g - iu$  has no inverse in  $A$ . Therefore  $(g - iu)^* = g + iu$  has no inverse, whence it lies in some maximal ideal  $y$ . Thus  $(g + iu)(y) = 0$  or  $g(y) = -i$ .

Since for any real positive  $N$ ,

$$\begin{aligned}(g + Niu)(x) &= i(1 + N), \\ (g - Niu)(y) &= -i(1 + N),\end{aligned}$$

we have

$$1 + N \leq \|g + Niu\|, \|g - Niu\|,$$

$$(1 + N)^2 \leq \|g + Niu\| \|g - Niu\|.$$

By (1), the right member

$$= \|g^2 + N^2u\| \leq N + N^2,$$

where we have now selected  $N > \|g^2\|$ .

The contradiction  $(1 + N)^2 \leq N + N^2$  shows that  $b + d = 0$ . Applying the same argument to  $if$  shows that  $a = c$ , whence  $f^*(x) = \overline{f(x)}$ .

<sup>1</sup> Gelfand, I., and Neumark, M., "On the Imbedding of Normed Rings into the Ring of Operators in Hilbert Space," *Rec. Math.*, 12 (54), 197-213 (1943).

## PREVENTION OF ADRENAL CORTICAL CARCINOMA BY DIETHYLSTILBESTROL\*. †

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Communicated August 8, 1946

It has been observed that adrenal cortical carcinomas, normally expected in JAX strain ce mice when gonadectomized soon after birth, did not appear following treatment of such mice with a synthetic estrogenic hormone, diethylstilbestrol.

In the present experiments fusion pellets, 25 per cent diethylstilbestrol in cholesterol, av. wt. 4.8 mg., were used.<sup>1</sup> These were introduced into the subcutaneous tissue of the right axilla when the mice were approximately 7 weeks of age. Only one pellet was used at this age in each mouse and none were implanted later in life.

Experimental mice were prepared by removing the gonads of strain ce mice immediately after birth. Adrenal cortical carcinomas had previously been observed in 100 per cent of gonadectomized female mice of this strain 6 months of age and older.<sup>2</sup> In gonadectomized male mice these tumors also had occurred starting at 7 months.<sup>3</sup>

*Diethylstilbestrol treated mice of both sexes observed up to 14 months of age did not have these tumors (table 1).*

Diet and other environmental factors were kept as near constant as possible in all groups.

Although time has not been sufficient for protection to be observed for mice of extremely advanced ages, the present results are encouraging for continued protection, since nodular hyperplasia of the adrenal cortex which preceded the occurrence of carcinomas in previous experiments was also prevented by the use of diethylstilbestrol pellets.

Further study needs to be made to find a protective agent for tumors of the adrenal cortex which will not at the same time cause other serious body disturbances. In the present experiments pituitary tumors of the type caused by estrogenic hormones were observed in the diethylstilbestrol treated mice as early as 7 months of age.

The problem of whether the presence of pituitary tumors is related to the direct tumor inhibitory action of stilbestrol on the adrenal cortex or whether stilbestrol impairs pituitary action on the adrenal cortex depends for its solution upon further investigation now being planned.

The possibility that the inhibitory action may result from certain effects of a grossly unchanged pituitary must also be considered.

TABLE 1  
EFFECT OF DIETHYLSTILBESTROL IN PREVENTING THE OCCURRENCE OF ADRENAL CORTICAL CARCINOMA

MONTHS OF AGE AT AUTOPSY	GONAECTOMIZED FEMALES				GONAECTOMIZED MALES			
	NON-TREATED TOTAL NO.	STILBESTROL TREATED NO. WITH TUMOR	NON-TREATED TOTAL NO.	STILBESTROL TREATED NO. WITH TUMOR	NON-TREATED TOTAL NO.	STILBESTROL TREATED NO. WITH TUMOR	NON-TREATED TOTAL NO.	STILBESTROL TREATED NO. WITH TUMOR
2	2	0	1	0	4	0	1	0
3	3	0	1	0	2	0	1	0
4	4	0	1	0	4	0	1	0
5	1	0	1	0	1	0	1	0
6	5	5	0	0	5	0	0	0
7	4	4	2	0	2	2	2	0
8	1	1	1	0	1	1	1	0
9	2	2	1	0	6	3	1	0
10	2	2	1	0	4	4	0	0
12	6	6	1	0	5	5	1*	0
14	2	2	1	0	1	1	1	0

\* This animal was a little under 12 months of age.

The effectiveness of stilbestrol as a substitute for natural gonadal secretion in preventing adrenal cortical tumor formation is, however, clear.

\* This work has been aided by grants to the Roscoe B. Jackson Memorial Laboratory from the Commonwealth Fund, The Anna Fuller Fund, The International Cancer Research Foundation, The Jane Coffin Childs Memorial Fund and The National Advisory Cancer Council.

† Technical assistance of Miss Margaret M. Dickie is gratefully acknowledged.

<sup>1</sup> These pellets were kindly prepared by Dr. Michael B. Shimkin, National Cancer Institute, Bethesda, Md.

<sup>2</sup> Woolley, G. W., and Little, C. C., *Cancer Research*, 5 (4), 193-202 (1945).

<sup>3</sup> Woolley, G. W., and Little, C. C., *Ibid.*, 5 (4), 211-219 (1945).

## THE GENETIC CONTROL OF GROWTH METABOLISM\*

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Communicated July 17, 1946

In analyzing the effects of genes on development, one basic approach is a determination of the effects of a gene on the metabolic activity of a particular group of cells as reflected by their rate of oxygen consumption. Goldschmidt suggested years ago<sup>1</sup> that genes produce their effects in development by altering the rate of chemical reactions, and more recently the idea that each gene regulates a particular step in a particular biosynthesis has gained favor.<sup>2</sup> In most animals it is impossible to locate exactly the cells which will give rise to a particular structure, but in *Drosophila* each adult organ develops from a discrete group of cells, an imaginal disc, which can easily be dissected out of the larva. The development of the Cartesian diver ultramicrorespirometer<sup>3</sup> provides a means for determining the oxygen consumption of very small pieces of tissue such as these imaginal discs.

A study was undertaken of the oxygen uptakes of the imaginal discs of wild type and certain mutant stocks of *Drosophila*. From these one can determine the effect of the substitution of a mutant gene for its wild type allele on the metabolism of the particular group of cells which give rise in the adult to the morphologically altered structure. Thus one more fact can be added to our knowledge of the relation between gene and phenotype. This paper gives the results of a study of the metabolism of wing and leg discs of normal flies and of certain mutant stocks with wings of reduced size. Other experiments are under way to test the effects of the "growth rate" genes, *dachs*, *dachsous*, *four-jointed*, and *combgap*, and the homoeotic genes, *bithorax*, *tetraltera*, and *aristapedia*, on the metabolism of the imaginal discs involved. It is possible to adapt the Cartesian diver apparatus to test for certain enzymes and coenzymes<sup>4</sup> and in later experiments it may be possible to identify the enzyme system affected by the mutant gene.

*Methods.*—The stocks of *Drosophila melanogaster* used in the present study were an isogenic wild stock established previously<sup>5</sup> and the mutant stocks "vestigial" (*vg*, chromosome 2, locus 67.0) and "miniature" (*m*, chromosome 1, locus 36.1). Miniature produces wings which are shorter and narrower than normal but of approximately normal shape. Vestigial wings are reduced to small stumps, which are held outstretched. Adult miniature wings are about two-thirds normal size and vestigial wings are less than one-quarter normal size.

Two types of imaginal discs were studied, the ventral mesothoracic disc,



which gives rise to the mesothoracic leg and a part of the ventral mesothoracic wall, and the dorsal mesothoracic disc, which becomes the wing and dorsal mesothoracic wall. In the late larva, the dorsal mesothoracic disc has a clearly marked circular region in its posterior part, called the wing bud, from which develops the wing.

Larvae and prepupae were dissected in a drop of phosphate-Locke's solution<sup>6</sup> (pH 7.4), the desired disc was removed and transferred by means of a calibrated braking pipette described by Holter<sup>7</sup> to the diver. The

TABLE 1  
RESPIRATION OF IMAGINAL DISCS

DISC	STOCK	AGE	NO. OF DETERMI- NATIONS	AV. OXYGEN UPTAKE PER DISC PER HR. ± S.E., MM. <sup>3</sup> /HR.	AV. DRY WEIGHT OF DISC ± S.E., μG.	O <sub>2</sub> , MM. <sup>3</sup> O <sub>2</sub> / HR./MG.
Wing	Wild	1 hr. after pupation	3	0.028 ± 0.0016	1.35 ± 0.03	20.7
Wing	<i>m</i>	1 hr. after pupation	2	0.025 ± 0.0011		18.5
Wing	<i>vg</i>	1 hr. after pupation	2	0.013 ± 0.0007		9.2
Wing	Wild	At pupation	2	0.026 ± 0.002	1.32 ± 0.04	19.5
Wing	<i>m</i>	At pupation	2	0.024 ± 0.0009		18.1
Wing	<i>vg</i>	At pupation	2	0.012 ± 0.0007		8.8
Wing	Wild	2-4 hrs. before pupation	4	0.020 ± 0.0005	0.95 ± 0.03	20.5
Wing	<i>m</i>	2-4 hrs. before pupation	4	0.019 ± 0.0005		19.5
Wing	<i>vg</i>	2-4 hrs. before pupation	3	0.009 ± 0.0003		9.8
Wing	Wild	5-10 hrs. before pupation	4	0.017 ± 0.0008	0.90 ± 0.04	19.1
Wing	<i>m</i>	5-10 hrs. before pupation	4	0.016 ± 0.001		17.3
Wing	<i>vg</i>	5-10 hrs. before pupation	5	0.008 ± 0.0013		9.5
Leg	Wild	1 hr. after pupation	4	0.012 ± 0.0017	0.58 ± 0.03	20.1
Leg	<i>m</i>	1 hr. after pupation	4	0.013 ± 0.002		22.7
Leg	<i>vg</i>	1 hr. after pupation	2	0.012 ± 0.002		20.1
Leg	Wild	2 hrs. before pupa- tion	7	0.0094 ± 0.0006	0.49 ± 0.04	19.1
Leg	<i>m</i>	2 hrs. before pupa- tion	3	0.0086 ± 0.0006		17.6
Leg	<i>vg</i>	2 hrs. before pupa- tion	4	0.0094 ± 0.001		19.1

divers had a total volume of 8 cu. mm. and contained 1.1 cu. mm. phosphate-Locke's solution in the bulb and 0.9 cu. mm. alkali and 0.9 cu. mm. oil seals in the neck. The "diver constant"<sup>8</sup> of the divers used was  $7.8 \times 10^{-4}$ . All respiration determinations were made at a temperature of 26.8°C.

The quartz fibre balance described by Lowry<sup>9</sup> was used to obtain the weights of the discs. Discs were rinsed several times in glass redistilled

water to remove the phosphate-Locke's solution and transferred to a quartz loop in a drop of distilled water. They were dried in an oven at 100°C. for 30 minutes and then weighed. The hooks were cleaned and reweighed to check the zero point. The balance was calibrated by putting known volumes of standard salt solutions on the hooks, drying and weighing.

**Results.**—The data of the experiments are given in table 1. At each stage of development the rate of oxygen consumption of the wild type wing discs and of the leg discs of all stocks used varies only slightly from 20 cu. mm.  $O_2$  per hour per milligram of tissue. The  $Q_{O_2}$  of the miniature wing discs at each stage of development is slightly less than that of the wild type discs and averages about 18 cu. mm.  $O_2$  per hour per milligram

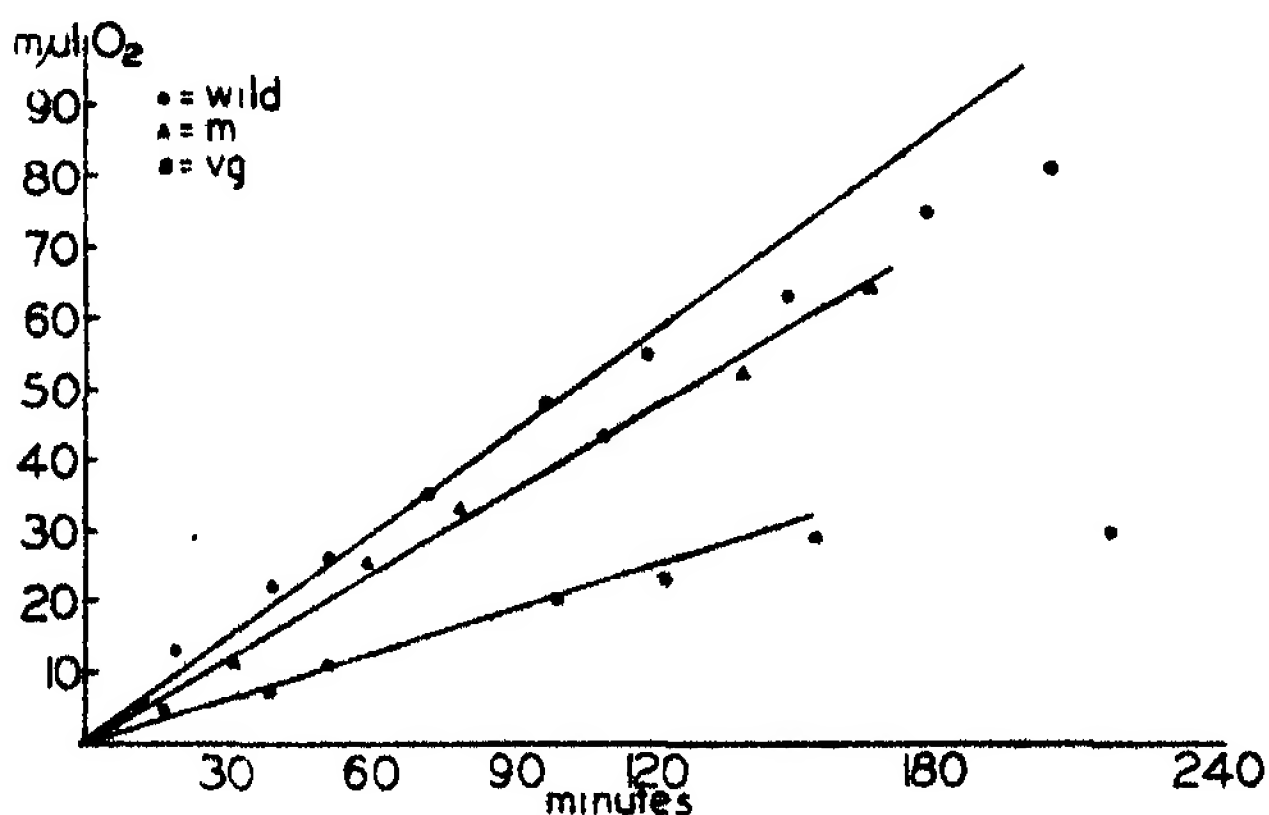


FIGURE 1.

Oxygen uptake in millimicroliters of wild type, vestigial and miniature wing discs dissected out 1 hour after pupation.

of tissue. The differences, though slight, are in the same direction and of about the same magnitude at each stage of development tested and are significant. The  $Q_{O_2}$  of the vestigial wing discs is less than half that of the wild type discs and averages about 9 cu. mm.  $O_2$  per hour per milligram of tissue. Both the weight of the wing discs and the rate of oxygen consumption per disc increase sharply in the two-hour period before pupation, but since they increase proportionally, the  $Q_{O_2}$  remains constant. Previous embryological studies<sup>10</sup> have shown this period to be one in which a great deal of morphological differentiation occurs.

In all the determinations of oxygen consumption, the experiments were continued from 120 to 240 minutes. The rate of oxygen uptake of the discs was constant for the first 120 minutes or so of each experiment but decreased slightly thereafter (Fig. 1). The discs contain considerable

reserves of substrate and will respire in the divers for twelve hours or more. One wild type wing disc left in a diver overnight consumed so much oxygen that a reading could not be taken, but it had used at least 450 m $\mu$ l. of oxygen.

*Discussion.*—The development of the Cartesian diver apparatus and the quartz fibre balance and the peculiarities of the mode of development of *Drosophila* and other Diptera afford a new approach to the study of gene action. It is possible with this technique to study the effects of particular genes on the rate of metabolism and on the enzyme constitution of the groups of cells which give rise to particular adult organs. Since each gene is believed to function by affecting the nature or quantity of a particular enzyme-controlled biosynthesis, this permits a new attack on the problem of gene physiology. The present study shows that the mutant genes affecting wing size produce their effects by altering the rate of some chemical reaction in the wing disc of the larva which is reflected in a lowered rate of oxygen consumption. These results do not mean that the *m* and *vg* genes affect the same chemical reaction in development, but simply that in affecting different processes they each lower the over-all metabolic rate of the disc. It is interesting to note that the metabolism of the leg discs and probably of the other discs of vestigial larvae is not changed, although the cells, of course, contain *vg* genes. The vestigial gene therefore produces its physiological as well as its morphological effects only in certain cells of the body, presumably due to the interaction of the gene or gene products with specific components of the cytoplasm of those cells.

The marked increase in the rate of oxygen uptake and in the weight of the wing discs which occurs in the two hours before pupation correlates closely with the rapid differentiation of the discs at that time found by morphological studies. The time of pupation is clearly marked by the eversion of the anterior spiracles and the cessation of motion of the larva; the age of the fly relative to this can be determined by inspection. The wing discs are growing more rapidly than the leg discs in the period between two hours before and one hour after pupation. The wing discs show a 43 per cent increase in oxygen uptake and a 42 per cent increase in weight in this period and the leg discs have only a 24 per cent increase in oxygen uptake and an 18 per cent increase in weight. This may be correlated with the morphological finding<sup>11</sup> that the wing buds are more advanced than the leg buds at pupation and have invaginated into the body cavity.

The weights of the wing discs of wild, miniature and vestigial flies taken at corresponding ages were very similar and showed the same range of variation, so they were averaged together (table 1). This finding corroborates Auerbach's statement<sup>12</sup> that *vg* wing discs are the same size as wild

type discs but show less differentiation at corresponding ages. Chen<sup>13</sup> had previously stated that vestigial discs were smaller than wild discs of corresponding ages.

*Summary.*—The rate of oxygen uptake per gram dry weight of the wing and leg discs of wild type, "miniature" and "vestigial" *Drosophila* was determined by the Cartesian diver ultramicrorespirometer. Miniature produces a slight and vestigial a marked decrease in the oxygen consumption of the wing disc but neither one affects the respiration of the leg discs. The mutant genes therefore produce their physiological as well as their morphological effects only in certain cells of the body, presumably by an interaction of the genes or gene products with specific cytoplasmic constituents of those cells.

\* I am indebted to Drs. E. G. Ball and C. B. Anfinsen for their helpful suggestions and advice in the course of this work, and to C. Lloyd Claff for the use of the Cartesian diver apparatus.

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<sup>12</sup> Auerbach, C., loc. cit.

<sup>13</sup> Chen, T. Y., loc. cit.

## LEAFHOPPER TRANSMISSION OF CORN STUNT\*

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A new corn disease prevalent in the Rio Grande Valley of Texas and the San Joaquin Valley of California has been under study since June, 1945, when affected plants were first received from Dr. A. A. Dunlap of the Texas Agricultural Experiment Station, College Station, Texas. The disease caused yellows-type symptoms and resembled, superficially, yellow stripe of corn, transmitted by the leafhopper *Peregrinus maidis* (Ashm.).<sup>1</sup> However, the conspicuous cell inclusion bodies associated with that disease were not found in the plants from Texas and California.

The disease was described from California by Frazier<sup>2</sup> under the name "streak" in March of last year. Altstatt,<sup>3</sup> a little later in the season, described it from Texas. Since the name "streak" already had been applied to another disease of corn, transmitted by the leafhopper *Cicadulina mbila* Naude,<sup>4</sup> the writer designated the new disease as "stunt" in reference to the shortening of internodes and the general stunting it caused. From the descriptions of Frazier and Altstatt and from observations on symptoms, it was concluded that stunt had not been described previously. It also was concluded that Frazier and Altstatt probably were correct in suggesting that it might be a virus disease.

Efforts to transmit stunt by the rubbing method with juice from diseased plants were unsuccessful. Also, attempts to transmit it by dodder, *Cuscuta campestris* Yuncker, failed. Frazier stated that high populations of the leafhopper *Baldulus maidis* (De L. and W.) were frequently found in fields where the disease occurred. Hence an effort was made to transmit stunt by this insect. A virus-free colony, obtained by taking newly hatched nymphs from a diseased corn plant before they had an opportunity to feed, was maintained on healthy corn plants and used in all tests reported below. The tests were carried out with potted plants in large, wood and screen cages and in lantern globe cages in greenhouses.

In one transmission experiment 14 healthy sweet corn plants of the variety Golden Bantam were exposed for 4 days to approximately 150 adult leafhoppers that had been hatched and reared on a diseased corn plant. Fourteen other corn plants of the same age and variety were exposed to about the same number of virus-free adult leafhoppers for 4 days. All of the plants exposed to the first colony came down with stunt in from 5 to 6 weeks after exposure. All of the plants exposed to the control colony remained healthy during a three-month period that they were kept under observation.



In another experiment 7 of 16 New Jersey No. 2 hybrid field corn plants, exposed for one day to a colony of 200 adult leafhoppers that had been hatched and reared on a diseased plant, came down with stunt in from 34 to 38 days after exposure. All of 16 similar plants of the same hybrid exposed to 200 virus-free leafhoppers for one day remained healthy during a period of 85 days that they were under observation.

In still another experiment 15 virus-free leafhoppers were allowed to feed on a diseased corn plant for 5 days and were then transferred to 26 healthy New Jersey No. 2 hybrid field corn plants. Fifteen other virus-free leafhoppers were allowed to feed on a healthy corn plant for 5 days and were then transferred to 26 other healthy corn plants of the same hybrid. After feeding on the two sets of plants for 30 days both groups of insects were removed and destroyed. In from 28 to 40 days thereafter 15 of the 26 plants exposed to the insects that had fed on the diseased corn plant, came down with stunt. The other 11 plants and the 26 control plants remained healthy during a period of 60 days that they were kept under observation.

These experiments and other similar experiments have proved that corn stunt is readily transmitted by *Baldulus maidis*.

\* The writer is indebted to Drs. A. A. Dunlap and G. E. Altstatt for specimens from Texas and to Dr. N. W. Frazier for diseased plants from California; he is also indebted to Dr. P. W. Oman for identifying the leafhopper used.

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## SOME PROPERTIES OF A GENETIC CYTOPLASMIC FACTOR IN *PARAMECIUM*\*

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Communicated August 8, 1946

Recent work by Sonneborn,<sup>1, 2, 3</sup> Lindegren,<sup>4</sup> Spiegelman<sup>5</sup> and L'Héritier and co-workers<sup>6, 7, 8</sup> has focused attention on the rôle of the cytoplasm in the determination and inheritance of characters. The present paper deals with the same subject from a new point of view and using new techniques. As a consequence it has been possible to estimate the number of particles of a particular kind of cytoplasmic factor in *Paramecium aurelia* and to study the rate at which the particles increase while the paramecia themselves are reproducing by fission at a different rate. These studies turned out not only to be of interest in themselves, but also to bring out certain

remarkable facts concerning the inheritance of the character determined by the cytoplasmic factor. By taking advantage of the differential rate of increase of the particles of the cytoplasmic factor in relation to the rate of fission of the paramecia, it became possible, on the one hand, to free the paramecia from the particles and so produce strains permanently lacking them and the character they control; and, on the other hand, to induce cultures which had lost the character because of considerable reduction (but not total loss) of their particle number, to reacquire the normal number of cytoplasmic factor particles and the character associated with them. Finally a technique was devised which demonstrated that a single cytoplasmic factor particle in an animal is sufficient to give rise to the normal number of particles.

Our knowledge of cytoplasmic factors in *P. aurelia* began with Sonneborn's<sup>1</sup> investigation of the determination and inheritance of the character known as "killer." Organisms of this sort alter the fluid in which they live so as to make it poisonous to non-killer or "sensitive" stocks. The full genetic analysis has been carried out only for killers of variety 4 of this species. This killer character depends upon the presence of a cytoplasmic factor termed "kappa" which is maintained and increased only in the proper genetic background, namely in the presence of a dominant gene, *K*. If *K* is replaced by its recessive allele, *k*, kappa disappears in the course of a few fissions and the resulting clone lacks the killer character; in fact, it becomes sensitive to the action of killers. If the gene *K* is reintroduced into such a sensitive, the killer character does not reappear. Gene *K* cannot initiate the production of kappa, it can only control the maintenance and increase of kappa when some is already present. The killer character can be restored to sensitives with gene *K* by reintroducing some cytoplasm from a killer during conjugation, and kappa and the restored character are thereafter maintained permanently during reproduction.

Four killer stocks of *P. aurelia* (stocks *G*, *H*, 36 and 50) have been employed in this study. They all belong to variety 2, a member of the same group of varieties as the variety 4 reported on by Sonneborn. (For an account of the varieties of *P. aurelia* see Sonneborn and Dippell.<sup>9</sup>) Variety 2 is sexually isolated from the other varieties. Each of these four killers and the variety 4 killers differ in the manner in which they affect sensitive animals. The action of the variety 4 killers and certain of the variety 2 killers has been described by Sonneborn.<sup>1, 10</sup>

The determination and inheritance of these killer characters in variety 2 has not been reported except for Sonneborn's<sup>1</sup> remark that the observations thus far made agree with the findings in variety 4 and indicate the presence of cytoplasmic factors. This remark (Sonneborn, personal communication) is based simply on the observation that in the  $F_1$  from a cross

of stock *G* by stock *H*, the two members of each conjugant pair produced clones with killer characters like the parent from which they derived their cytoplasm. This result is comparable to Sonneborn's<sup>1</sup> results in crosses of variety 4 killers to sensitives, in which the killer conjugant produced a killer clone, the sensitive conjugant a sensitive clone. In view of what is known further in variety 4, it therefore seems likely that cytoplasmic factors are also involved in the determination of the killer characters in variety 2. The word "kappa" will be used here as a general term to refer also to the various cytoplasmic factors associated with killing in variety 2. It is not meant to imply that these factors are identical in the various killer stocks.

*The Control of Killing in Variety 2.*—Soon after this study was begun a fundamental difference between the variety 2 and variety 4 killers became apparent. Killer stocks in variety 2, in striking contrast to those in variety 4, commonly give rise to non-killer, sensitive progeny. An investigation of the factors responsible for this production of sensitives revealed the following: (1) When animals are fed only a small amount of food so that a slow rate of fission is maintained (one-half fission per day, for example) all stocks remain pure killer indefinitely. On the other hand, when more food is made available and the rate of fission is increased, cultures of all four killer stocks lose their ability to kill and then become sensitive. Loss of killing is accomplished in 6 to 8 fissions at 3 fissions per day. The maximum rate of fission at which the stocks can grow and remain pure killer is apparently greatest for stock *G*. (2) Non-killer and sensitive cultures produced in this manner will revert back to killer after a few days when their growth is slowed or stopped, unless the fast growth has been maintained beyond a certain critical point. (3) The longer and the faster the rapid fissions are maintained, the longer it takes the animals in the cultures to revert to strong killers. (4) If rapid fissions are maintained for long enough, killing will never return. There seems to be but one reasonable explanation for these four facts. The hereditary basis for killing, kappa, fails to keep pace with the rate of growth of the animal; the amount per animal is progressively reduced in quantity by successive fissions; and finally animals are produced which have none at all.

*Estimation of the Number of Particles of Kappa and Their Rate of Increase.*—It should be possible to measure how long and how fast a strong killer has to be grown in order to rid it of kappa, and then to use this information to estimate the original number of particles and their rate of increase. An experiment designed to supply this information was started by isolating 192 of the progeny of a single original killer of stock *G* which had undergone eight rapid fissions at 27°C. Every time each isolation underwent 4 or 5 more fissions, one animal was transferred from each culture and a new set of 192 isolations was made. The remaining animals in each

of the 192 discard cultures left from each transfer continued for about 4 more rapid fissions when growth was suppressed to allow animals capable of reverting to killer to do so. It was then determined what proportion of each set of 192 discard cultures contained no killers. The proportion obtained for each set represents the proportion of isolated individuals which were incapable of producing killer progeny. If one assumes that one particle is sufficient to enable an animal to revert to killer and that particles are neither lost nor destroyed, then this proportion of animals producing no killer progeny is also the proportion of animals with no particles. Evidence in support of these assumptions is given in the next experiment. The data showed that after the original killer had undergone 12 fissions (in 3.6 days) the proportion of the 192 animals producing no killer progeny was 0.01; after 16 fissions (in 4.7 days), the proportion was 0.245; after 21 fissions (6.2 days), 0.74; after 26 (7.7 days), 0.93; after 31 (9.2 days), 0.97; and after 36 (10.7 days), 0.99.

If the distribution of the number of particles among the animals in the culture at any time is random, then the Poisson distribution can be used to determine the mean number of particles per animal from the observed proportion of animals with no particles. This can be done by making use of the first term of the Poisson series which relates the number of individuals with no particles ( $P_0$ ) to the mean number of particles per animal ( $m$ ) by the equation  $P_0 = e^{-m}$  (where  $e$  is the base of natural logarithms). The applicability of the Poisson distribution, however, depends upon the condition that kappa is distributed randomly as discrete particles. Evidence that this is the case will appear subsequently. To Prof. H. J. Muller of Indiana University the author is indebted for pointing out that a correction must be introduced into the Poisson series to account for the slight non-randomness introduced by the increase in number of the particles as the animals increase by fission. For the present, we will neglect this correction, and later on consider its magnitude. After 16 fissions, when  $P_0$  was 0.245, it can be calculated, using the relation  $P_0 = e^{-m}$ , that  $m$  was 1.4; and after 21 fissions, when  $P_0$  was 0.74,  $m$  was 0.30. But in going from 16 fissions to 21 fissions each animal produced 32 animals and its mean particle number of 1.4 must have produced  $0.30 \times 32 = 9.6$  particles. If 1.4 particles produced 9.6 particles, then 1 particle produced  $9.6/1.4 = 6.9$  particles. So the rate of increase of the particles may be expressed by saying that one particle produces 6.9 particles while the animals are going through 5 fissions in 1.5 days. Or it can be converted and expressed as 1.9 duplications per day for the particles while the animals are undergoing 3.3 fissions per day. This rate can now be used to predict the mean number of particles and also  $P_0$  for any other fission, assuming the rate is constant. For example, the mean can be calculated to be 0.065 after the 26th fission, and this leads to a value of 0.94 for  $P_0$  as compared



with an observed value of 0.93. All predicted values give good agreement, indicating that the assumption of the constancy of the rate is valid and also supporting the assumption of random distribution of discrete particles of kappa. If one assumes that the rate of 1.9 duplications per day has been constant during all the fissions back to the start of the experiment, the number of particles in the original killer (mean number after 0 fissions) can be computed in a similar manner. This number varies very slightly with the particular method used to make the calculation, but good agreement is obtained with a number of 180 particles. It is considered likely that the rate is probably somewhat less than 1.9 for the first few fissions, but it is thought that the error introduced into the estimate of 180 particles is probably small.

It was indicated above that an error was introduced into the calculations by the fact that the Poisson distribution does not fit the situation exactly. An approach has been made to this problem by a purely empirical method. A "pencil and paper" trial was run starting with a hypothetical animal with 180 particles. The particles were divided at random (use was made of Tippett's<sup>11</sup> "random sampling numbers") into two groups corresponding to a fission of the animal, and this was repeated for each fission. After 12, 16, 21, etc., fissions the number with no particles was compared with the experimental data. It was also necessary, of course, to increase the number of particles between each fission to simulate the calculated rate of 6.9 particles from 1, each 5 fissions (or 2.8 duplications for 5 fissions or 0.56 duplications for 1 fission). Actually, agreement was not good when a rate of 1.9 duplications per day and a particle number of 180 was used, due to the fact that these values were calculated on the basis of the Poisson distribution which is not strictly applicable. Different trials, however, showed excellent agreement if the rate was taken as 1.9 and the particle number as 256. These last values are thus considered to be the best solution to the problem.

*Demonstration That a Single Particle of Kappa Is Sufficient to Enable an Animal to Revert to Killer.*—An experiment was designed to test the assumption that one particle is all that is necessary to enable an animal to revert to killer under conditions of arrested growth. A number of rapidly dividing animals were originally isolated into separate containers and allowed to go through various numbers of fissions; all of the progeny of each original animal were kept in the container to form a culture. If the assumption is correct and no appreciable number of particles is lost or destroyed, then we would expect that any original animal containing a particle would give rise in the culture derived from it to at least some animals capable of reversion to killer. So that if, for example, 10% of the original animals had no particles, then 10% of the cultures would have no particles and hence no animals capable of reversion. Furthermore, this percentage



should be independent of the number of rapid fissions each original animal went through after isolation in the container. On the other hand, if more than one particle is necessary, then the percentage of the original animals giving rise to exclusively permanent sensitive progeny should increase as successive fissions cause the number of particles in *all* the progeny to drop below the number necessary for reversion.

The critical experiment was done starting originally with animals of stock *G* whose number of particles had been reduced by a series of rapid fissions. (These animals were obtained from a single killer which was allowed to go through 16 fissions at a rate of 3.3 fissions per day.) A number of these original animals were then removed individually to separate containers and allowed to undergo different numbers of rapid fissions at a rate of 3 fissions per day; then growth was stopped and it was determined whether *any* of the progeny of each of the original animals reverted to killer. It was found that 10.9% (14 out of 128) of the original animals after 8 rapid fissions gave rise to cultures of exclusively permanent sensitive progeny. And 10.2% (13 out of 127) of the original animals after 15 rapid fissions gave rise to exclusively permanent sensitive progeny. The fact that the percentage was not greater following 15 fissions than following 8 fissions is in agreement with the assumption that one particle is sufficient for reversion.

*Summary and Discussion.*—It has been shown that the cytoplasmic factor responsible for the killing phenotype in stocks of variety 2 of *P. aurelia* cannot increase as fast as the animals can be grown. By taking advantage of this differential growth rate it has been possible to reduce the number of particles of the cytoplasmic factor until the ability to kill is lost, and even to free animals of particles completely. Likewise, it has been established that animals which have been reduced to only a single particle will regain their normal number and become killers again when the fissions are slowed or stopped entirely. A study of the time and fission rate required to free animals of the particles has made it possible to estimate the number of particles as approximately 256 in one particular individual and also to calculate that the duplication rate of the particles was about 1.9 times per day while the paramecia were dividing 3.3 times per day.

It has been noted that the variety 4 killers never lose their killing power no matter how fast they are grown, as was pointed out by Sonneborn.<sup>2</sup> This is an important difference between the two varieties.

Sonneborn<sup>2, 3</sup> has discussed the possibility that the cytoplasmic factor, kappa, is bound to the gene *K* in the macronucleus where it is reproduced as a part of the gene. He has also considered that it can only pass from the nucleus into the cytoplasm at nuclear reorganization when the macronucleus breaks down. The reduction in number of particles by rapid growth described here for variety 2 is not in agreement with this view as

stated for variety 4, for in variety 2 the particles can be removed from the animals without the occurrence of macronuclear breakdown. In order to make this hypothesis fit the variety 2 situation the assumption would have to be made that in variety 2, unlike variety 4, the particles readily dissociate from the macronucleus and pass into the cytoplasm during vegetative reproduction.

L'Héritier and Tessier<sup>6, 7</sup> have reported on a "genoid" or cytoplasmic factor determining sensitivity to carbon dioxide in *Drosophila*. L'Héritier and Sigot<sup>8</sup> have shown that when small amounts of the cytoplasmic factor are introduced along with the sperm into the egg at fertilization, the inhibitory effect of extreme temperature reduces the rate of increase of the cytoplasmic factor to less than the division rate of the embryonic cells. They have shown that this results in the production of many cells lacking the cytoplasmic factor. This situation is remarkably similar to the reduction of the number of particles of the cytoplasmic factor in the variety 2 killers which was also accomplished by differential growth rates.

The behavior of the cytoplasmic factor described here calls attention to the significant fact that self reproducing cytoplasmic components may not increase at a rate strictly correlated with the growth rate of the cell. They may increase faster or slower, depending upon conditions. This cannot only cause a great variation in the amount of these components and their associated effects, but it can even result in their complete and permanent loss.

I am most grateful to Dr. T. M. Sonneborn for his invaluable suggestions and to his staff for technical assistance.

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## *ON THE SINGULAR SOLUTIONS OF CERTAIN DIFFERENTIAL EQUATIONS OF THE SECOND ORDER*

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1. We consider a differential equation of the form

$$F(y'', y, x) = 0 \quad (1)$$

in which  $F$  is a polynomial in  $y''$  and  $y$  whose coefficients are functions of  $x$  which are analytic in some area. We assume  $F$  to be algebraically irreducible in a given differential field containing its coefficients. The singular solutions of (1) are those which annul  $\partial F / \partial y''$ . In studying a singular solution, it is legitimate to assume the solution to be given by  $y = 0$ . In the particular case in which  $y = 0$  does not belong to the general solution of (1),  $F$ , considered as a polynomial in  $y''$  and  $y$ , contains a term in  $y$  alone, that is, a term free of  $y''$ , which is of lower degree than every other term of  $F$ .<sup>1</sup> If then we solve (1) for  $y''$  as a function of  $x$  and  $y$  for the neighborhood of  $y = 0$ , we obtain a set of expansions for  $y''$  of the type

$$y'' = \sum_{i=0}^{\infty} \alpha_i(x) y^{(p+i)/r}, \quad (2)$$

where, in each expansion,  $p$  and  $r$  are positive integers with  $p < r$  and  $\alpha_0(x)$  is not identically zero. Naturally,  $p$  and  $r$  may be different for different expansions of the set.

Adopting now a more general point of view, we start with a differential equation given by (2), with the second member a series as just described, not necessarily derived from an algebraic differential equation. It is understood then that  $p$  and  $r$  are positive integers with  $p < r$  and that,  $v$  representing  $y^{1/r}$ , the second member is analytic in  $x$  and  $v$  at some point  $x = a$ ,  $v = 0$ . Without loss of generality, we assume that  $a = 0$  and that  $\alpha_0(0) \neq 0$ .

We are going to show that  $y = 0$  is an envelope of two one-parameter families of solutions of (2), in general distinct from each other, but coincident in a very special case. Where a curve of one of the families contacts  $y = 0$ , the curvature of the curve is zero.

The problem treated here may be considered as coming under the theory of the movable singularities of a pair of equations of the first order. Malmquist<sup>2</sup> has written on this question, but his procedure does not lead to the result presented here.

2. We shall assume that  $p + r$  is even. Where this is not so at the start, it can be brought about by doubling the numerators and denominators in all exponents. On the other hand, we assume that, if, in the exponents effectively present, the numerators all have a factor greater than unity in common with  $r$ , that factor is 2 and  $(p + r)/2$  is odd.

We are going to find two differential equations

$$y' = \sum_{i=0}^{\infty} \beta_i(x) y^{(q+i)/r} \quad (3)$$

with  $q = (p + r)/2$ , whose solutions are solutions of (2). For the solution of an equation (3) which vanishes at  $x = c$ , an expansion in powers of  $x - c$  is found by putting  $y = v^r$  in (3) and then considering  $x$  as a function of  $v$ . The existence theorem gives an expansion for  $x$  in terms of  $v$  which is readily inverted to give

$$y = \sum_{i=0}^{\infty} A_i(c) (x - c)^{(r+i)/(r-q)} \quad (4)$$

As  $r/(r - q) = 2r/(r - p) > 2$ , the solution  $y = 0$  of (2) is seen to be an envelope of the solutions of (3). For  $x = c$ , the first and second derivatives of  $y$  in (4) are zero.

3. We must equate the second member of (2) to

$$\sum_{i=0}^{\infty} \beta_i' y^{(q+i)/r} + r^{-1} \left[ \sum_{i=0}^{\infty} (q + i) \beta_i y^{(q-r+i)/r} \right] \left[ \sum_{i=0}^{\infty} \beta_i y^{(q+i)/r} \right]. \quad (5)$$

We put  $h = q - p = r - q$ . From (2) and (5), we obtain equations for the  $\beta_i$  which change in form when  $i$  reaches the value  $h$ .

We have

$$\beta_0^2 = r q^{-1} \alpha_0, \quad \beta_0 \beta_1 = r(2q + 1)^{-1} \alpha_1. \quad (6)$$

For an even value of  $i$  less than  $h$ , with  $i = 2j$ ,

$$2\beta_0 \beta_i + 2\beta_1 \beta_{i-1} + \dots + 2\beta_{j-1} \beta_{j+1} + \beta_j^2 = 2r(2q + i)^{-1} \alpha_i. \quad (7)$$

For an odd value of  $i$  less than  $h$ , with  $i = 2j - 1$ ,

$$2\beta_0 \beta_i + 2\beta_1 \beta_{i-1} + \dots + 2\beta_{j-1} \beta_j = 2r(2q + i)^{-1} \alpha_i. \quad (8)$$



For  $i = h + t$  with  $t \geq 0$ ,

$$2\beta_0\beta_{h+t} + \dots = 2r(2q + h + t)^{-1}(\alpha_{h+t} - \beta_t'). \quad (9)$$

These equations determine the  $\beta_i$  in succession. We obtain two equations (3). In the special case in which the  $\alpha_i$  are all constants, the second members of the two equations will be negatives of each other, for the  $\beta_t'$  in (9) will all be zero.<sup>3</sup> If, in addition,  $r$  is even, the two second members, under the assumption that they are convergent, can be seen to represent the same  $r$ -valued function.

It remains to be proved that the second members of the equations (3) are convergent in the neighborhood of  $x = y = 0$ . This we do by a majorant process.

4. We write (2) in the form

$$y'' = f(x, v) \quad (10)$$

with  $v = y^{1/r}$ . Under the substitution  $x = ax_1$ ,  $y = b^r y_1$ ,  $v = bv_1$ , with  $a$  and  $b$  constants, (10) goes over into

$$y_1'' = a^2 b^{-r} f(ax_1, bv_1).$$

We may take  $a$  and  $b$  as small as we please. It is thus legitimate to assume that  $f(x, v)$  is analytic for  $|x| \leq 1$ ,  $|v| \leq 1$  and that  $\alpha_0(x)$  in (2) is distinct from zero for  $|x| \leq 1$ . The region of analyticity of  $f(x, v)$  gives that function a majorant of the form

$$Mv^2(1-x)^{-1}(1-v)^{-1}.$$

We shall take  $M$  so that  $M \geq 1$ . Each  $\alpha_j(x)$  in (2) will have  $M(1-x)^{-1}$  as a majorant. Then, *a fortiori*, for  $j \geq 2$ ,  $\alpha_j$  will have  $M(1-x)^{2-2j}$  as a majorant.

From (6) we see that  $\beta_0(x)$  is analytic, and distinct from zero, for  $|x| \leq 1$ . We may thus suppose  $M$  to be such that  $1/\beta_0(x)$  has  $M(1-x)^{-1}$  for majorant. We shall suppose also that  $\beta_0'$  and  $\beta_1$  have  $M(1-x)^{-1}$  for majorant.

5. We now consider the equation for an unknown function  $u$

$$u^2 + 4ry(1-x)^{1-2h}u + 4r(1-x)^{2-2h}y^{1+(q/r)}(M + M^{-1}) = M^{-2}(1-x)^2y^{2q/r} - 2M \sum_{i=0}^{\infty} y^{(2q+i+1)/r}(1-x)^{-2i}. \quad (11)$$

We seek a solution of (11) in the form

$$u = \sum_{i=0}^{\infty} \gamma_i(x)y^{(q+i)/r}. \quad (12)$$

We find that  $\gamma_0^2 = M^{-2}(1-x)^2$  and that, for  $0 < i < h$ ,

$$2\gamma_0\gamma_i + \dots = -2M(1-x)^{2-2i}.$$

We let  $\gamma_0 = -M^{-1}(1-x)$  and find for  $\gamma_h$  the equation

$$2\gamma_0\gamma_h + \dots = -(2+4r)M(1-x)^{2-2h}.$$

For  $\gamma_{h+t}$ , with  $t > 0$ , we find

$$2\gamma_0\gamma_{h+t} + \dots = -2M(1-x)^{-2(h+t-1)} - 4r\gamma_t(1-x)^{1-2h}.$$

The first member of the equation for any  $\gamma_t$  is obtained by replacing  $\beta$  by  $\gamma$  everywhere in the first member of the equation for the corresponding  $\beta_t$ .

For every  $j \geq 1$ , we find that  $\gamma_j = c_j(1-x)^{1-2j}$  with  $c_j$  a positive constant. We have

$$\gamma_1 = M^2(1-x)^{-1} \text{ or } \gamma_1 = (1+2r)M^2(1-x)^{-1}$$

according as  $h > 1$  or  $h = 1$ . In any case, because  $M(1-x)^{-1}$  is a majorant for  $\beta_1$  and  $M \geq 1$ , it follows that  $\gamma_1$  is a majorant for  $\beta_1$ .

Suppose that  $h > 2$ . We compare  $\beta_2$  and  $\gamma_2$ . As  $2q = p+r$ , we have  $2r/(2q+2) < 2$ . Using the fact that  $M(1-x)^{-1}$  is a majorant for  $1/\beta_0$ , we find  $\gamma_2$  to be a majorant for  $\beta_2$ . Continuing, we find  $\gamma_i$  to be a majorant for  $\beta_i$  for  $i = 1, \dots, h-1$ .

Let us compare  $\beta_h$  and  $\gamma_h$  for  $h > 1$ . We have arranged so that  $M(1-x)^{-1}$  is a majorant for  $\beta_0'$ . In addition,  $4r > 2r/(2q+h)$ . It follows that  $\gamma_h$  is a majorant for  $\beta_h$  when  $h > 1$ . We already know this to be true for  $h = 1$ .

We now carry out an induction, using any  $h > 0$ . Let  $t$  be any positive integer. Let us suppose that  $\gamma_{h+j}$  is a majorant for  $\beta_{h+j}$ , for  $j = 0, \dots, t-1$ . We shall prove that  $\gamma_{h+t}$  is a majorant for  $\beta_{h+t}$ .

We know that  $\gamma_t$  is a majorant for  $\beta_t$ . As  $\gamma_t = c_t(1-x)^{1-2t}$ , we see that

$$(2t-1)\gamma_t(1-x)^{-1}$$

is a majorant for  $\beta_t'$ . Because

$$4 > 2(2t-1)(2q+h+t)^{-1},$$

it follows that  $\gamma_{h+t}$  is a majorant for  $\beta_{h+t}$ .

As (11) is merely a quadratic equation for  $u$ , the series (12) which we have obtained represents a function of  $x$  and  $v$  which is analytic for  $x = v = 0$ . The same is then true for the second member of (3).

<sup>1</sup> Ritt, *Ann. Math.*, 37, 552 (1936).

<sup>2</sup> *Sur les points singuliers des équations différentielles*, *Arkiv mat., astr. fysik*, 27 (1921).

<sup>3</sup> When the  $\alpha_i$  are constants, the series in (3) are merely square roots of twice the integral with respect to  $y$  of the series in (2).

# FINITELY ADDITIVE SET FUNCTIONS AND STOCHASTIC PROCESSES

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We will randomize finitely additive set functions and in this way obtain a general type of stochastic process which includes most processes known, discrete or subdivisible. From our approach it is very easy to form Stieltjes-integrals with random functions of unbounded variation in connection with a new concept of stability; and to interpret Fourier analysis of random functions as an isomorphic replacement of one stochastic process by another equivalent one; usually, but not necessarily, of a continuous process by a discrete one.

At first assume that a probability is already given. We thus have at our disposal all Lebesgue measurable (almost everywhere) functions  $f(\lambda)$  on a measure space  $\{\lambda\}$  of total measure 1. Now take an arbitrary point set  $S$  and a collection of its subsets  $\{E\}$ . The collection shall be a complemented lattice  $B$ . With each  $E$  we associate one of our random functions  $f(E; \lambda)$  and we assume that for  $E_1, \dots, E_n$  disjoint we have

$$f(E_1 + E_2 + \dots; \lambda) = f(E_1; \lambda) + \dots + f(E_n; \lambda)$$

almost everywhere. Such a function  $f(E; \lambda)$  we consider to be a *stochastic phenomenon*. For instance, any sequence  $f_n(\lambda)$ ,  $n = 1, \dots$ , is such a phenomenon, if  $S$  is the sequence  $(1, 2, \dots)$  and  $E$  is an arbitrary finite subsequence with  $F(E; \lambda) = f_{n_1} + \dots + f_{n_k}$ . In this "discrete" case, the complemented lattice  $B$  is not also a Boolean algebra which contains  $S$  itself; if it were so, then this would roughly correspond to a process of finite duration, which an unending sequence usually is not.

A partition  $\delta$  shall be any finite number of disjoint elements  $E_1, \dots, E_n$  of  $B$ . A second partition  $\delta' = (E_m^1)$  is a subpartition of  $\delta$ ,  $\delta' > \delta$ , if each  $E_k$  is a union of one or several  $E_m^1$ , with perhaps some  $E_m^1$  left over altogether. With each  $\delta$  we associate the joint distribution function of the  $n$  functions  $y_k = f(E_k; \lambda)$ ,  $k = 1, \dots, n$ , and we introduce the  $n$ -dimensional Fourier transform (characteristic function) of that distribution, denoting the characteristic function by

$$\varphi_\delta(\alpha_1, \dots, \alpha_n). \quad (*)$$

Now, if  $\delta' > \delta$ , then the function  $\varphi_\delta(\alpha)$  arises from  $\varphi_{\delta'}(\alpha')$  by a certain inverse transformation

$$\alpha'_k = \sum_m c_{km} \alpha_m \quad k = 1, \dots, n$$

in which each  $c_{km}$  is either 0 or 1. These transformations constitute consistency conditions.

Now, conversely, we start from  $S$  and  $B$ , and we take any directed set of partitions  $\{\delta\}$ , no matter how small or how large, and we assume that corresponding to those partitions there exists a family of characteristic functions (\*) which are so consistent. Then, following Kolmogoroff, it can be shown that there exists a measure space  $\{\lambda\}$  and a stochastic phenomenon  $f(E; \lambda)$  which is compatible with (\*). For that reason, we look upon any collection of functions (\*) of the stated description as a *stochastic process* as such, and on any corresponding function  $f(E; \lambda)$  as its phenomenological realization.

A *homogeneous* process arises if there exists an additive weight (measure)  $\mu(E)$  on  $B$ —not to be confused with the probability measure on  $\{\lambda\}$ —and a function  $\psi(\alpha)$  such that

$$\varphi_\delta(\alpha_1, \dots, \alpha_n) = \prod_{r=1}^n \exp(-\mu(E_r)\psi(\alpha_r)).$$

Wiener's differential space arises if  $\psi(\alpha) = \alpha^2$ , and a more general Levy-space arises if  $\psi(\alpha) = |\alpha|^q$ ,  $0 < q \leq 2$ . Wiener has shown that, in his case,  $f(E; \lambda)$  has unbounded variation on  $B$  for almost all  $\lambda$ . We show that this also holds for  $1 \leq q \leq 2$ , thus in particular also for the Cauchy distribution  $q = 1$ , but that for  $0 < q < 1$  almost all  $\lambda$  do have bounded variation.

As soon as a weight  $\mu(E)$  exists, even if the process is not homogeneous, and if it has a  $\sigma$ -extension, we can define on  $S$  numerical  $L_p$ -functions relative to  $\mu(E)$ , for  $p \geq 1$ . For a step function  $h(x)$ , the Stieltjes integral

$$\zeta(\lambda) = \int_S h(x) dF(E; \lambda)$$

is defined as a finite sum, and we say that our process is  $L_p$ -stable,  $p \geq 1$ , if for any sequence of step functions which converge in  $L_p$ -norm, the integral converges in measure. If  $\mu(E)$  is bounded on  $B$ , then Wiener space is stable for every  $p \geq 2$ , and Levy space for  $p \geq \max.(1, q)$ . This is no longer so for unbounded  $\mu(E)$ , that is, for a process of infinite duration; however, even then the space is still  $L_q$ -stable,  $q > 1$ , and there are many  $L_2$ -stable processes which are not even homogeneous.

If  $\{h_n(x)\}$  is a complete orthogonal system on  $S$  we can form the expansion

$$\frac{df(x, \lambda)}{d_x} \sim \sum f_n(\lambda) h_n(x),$$

where  $f_n(\lambda) = \int h_n df$ , and the sequence of random variable  $\{f_n(\lambda)\}$  can be interpreted as a new discrete stochastic phenomenon, whose underlying process is a dual to the original one. Thus, in particular, a time-limited subdivisible differential (Wiener) space is dualized into another differential

space, the dual being a discrete one. However, for infinite duration the dualism is a self-dualism in the following sense.

*A Wiener differential process on the half line  $0 \leq x < \infty$  is its own cosine and sine transform, as a process.*

Finally from our approach we are able to analyze Khintchine's stationary processes very systematically, although none of the results can be strictly new, since these processes are more or less included in the theory of Hilbert space.

A full account will appear in another journal.

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## MUTATIONS INVOLVING THE REQUIREMENT OF URACIL IN *CLOSTRIDIUM*\*

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Some of the variations which occur during the growth of bacteria are due to the selection of types which arise at random by mutation. In the case of resistance to phage<sup>1</sup> and radiations<sup>2</sup> in *Escherichia coli* and penicillin resistance in *Staphylococcus aureus*<sup>3</sup> these changes behave like genic mutations in that there is a finite probability for each individual bacterium to undergo an hereditary change in the course of its lifetime. These changes are not induced by the unfavorable agents but occur in their absence. Phage radiation and penicillin simply select those resistant organisms which arise at random. With respect to nutritional requirements the demonstration of the genic nature of mutation is not so clear. Roepke, *et al.*,<sup>4</sup> found that mutants of *E. coli* with specific growth factor requirements appeared after x-radiation. However, they were unable to attribute the changes to the x-radiation because of the incidence of spontaneous mutations. This was also the experience of Gray and Tatum<sup>5</sup> with *E. coli* but these authors showed that growth factor deficiencies were induced in *Acetobacter melanogenum* by x-radiation. Later, when more material was available, Tatum<sup>6</sup> was able to show that x-radiation significantly raised the frequency of nutritional mutants in *E. coli*. By analogy with the production of biochemical mutations in *Neurospora*,<sup>7</sup> a sexual organism, the latter authors suggest that biosyntheses in bacteria are controlled by specific genes. We have been able to show that a strain of *Clostridium septicum* mutates to a condition where it is no longer able to synthesize the pyrimidine, uracil, but requires it in the medium for growth. Conversely, the uracil-dependent strain mutates to a state where uracil is



synthesized and need not be supplied. These mutations occur throughout the growth of the culture and even in the presence of uracil. Thus, these changes in nutritional requirement satisfy criteria for gene mutation which can be tested in an organism in which sexual reproduction has not been demonstrated.

Strain 59 Li of *Cl. septicum* will grow in a chemically defined medium which contains salts, sugar, purified Bacto-casamino acids, glutamine, tryptophane, cystine, cysteine hydrochloride and the vitamins pantothenic acid, nicotinic acid, pyridoxine, thiamin and biotin.<sup>8</sup> However, on this basal medium we found growth to be erratic and inconsistent among similar tubes in the same experiment.<sup>9</sup> The time of initiation of growth varied considerably and not infrequently growth failed to occur (table 1).

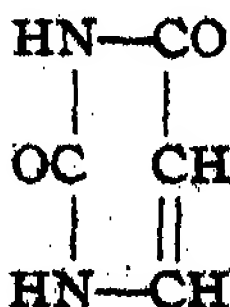
TABLE 1  
VARIATION IN THE ONSET OF GROWTH OF STRAIN 59 Li ON CHEMICALLY DEFINED MEDIUM

TUBE	APPEARANCE* OF CULTURES AT VARIOUS HOURS AFTER INOCULATION			
	8	22	34	51
1	—	—	—	—
2	—	—	+	+++
3	—	—	+	+++
4	—	—	—	—
5	—	+	+++	+++
6	—	+++	+++	+++
7	—	+	+++	+++
8	—	+++	+++	+++
9	—	+	+++	+++
10	—	—	+	+++
11	—	—	—	—
12	—	—	++	+++

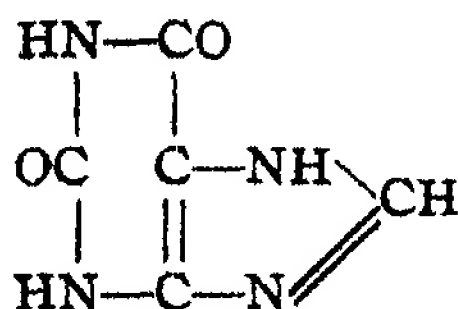
\* — designates no growth; increasing densities of bacteria are represented by +, ++ and +++.

But when growth did occur, irrespective of when it started, the rate of growth during the logarithmic phase and the final crop of bacteria were the same in similar tubes. The average time for one generation to be produced during the logarithmic phase of growth was  $80 \pm 4(\sigma_M)$  minutes. After growth was complete the cultures contained on an average  $13.0 \pm 1.0$  mg. of bacterial nitrogen per 100 ml.

It was found that this variation in the lag period could be eliminated by the addition to the medium of the pyrimidine, uracil,



or to a lesser extent by its related purine, xanthine,



(table 2). . In the presence of an optimum concentration of 10  $\gamma$  per ml. of uracil growth was completed in every case and generally in less than 18 hours. The rate of growth and the yield of bacteria were the same in the presence as in the absence of uracil (table 3). The role of uracil is in the reduction of the length of the lag phase and in the assurance that growth will occur.

TABLE 2

THE TIME IN HOURS REQUIRED BY STRAIN 59 Li TO REACH ALMOST COMPLETE GROWTH (+++) IN THE PRESENCE OF DIFFERENT COMBINATIONS OF PURINES AND PYRIMIDINES, EACH PRESENT IN THE CONCENTRATION OF 5  $\gamma$  PER ML. EACH TIME IS THE AVERAGE OBTAINED FROM 3 TO 8 CULTURES

	0	ADENINE	GUANINE	XANTHINE	URACIL	XANTHINE URACIL	GUANINE URACIL	XANTHINE GUANINE
0	43	55	43	30	21	..	..	..
Adenine	..	..	37	76	37	17	33	72
Guanine	..	..	..	39	25	24	..	..
Xanthine	..	..	..	..	19	..	24	..
Uracil	..	..	..	..	..	..	..	24

TABLE 3

THE EFFECT OF URACIL ON THE GROWTH OF URACIL-DEPENDENT STRAIN, 59 Li B

URACIL, $\gamma$ /ML.	MINUTES TO 2 MG. NITROGEN $\pm$ AVERAGE DEVIATION	GENERATION TIME, MINUTES	MG. NITROGEN AFTER 48 HOURS
0	1630 $\pm$ 500*	79	12.0
10	800 $\pm$ 162	78	12.5

\* This figure is the average of observations on six cultures from two different experiments. Of the remaining tubes in the first experiment, one grew during the third day after inoculation, one during the fourth day and three had not grown on the fifth day when they were discarded. In the second experiment, one culture grew during the fourth day, one during the fifth and one, which had not grown, was discarded on the fifth day. None of these very long lag periods were included in the average.

One explanation of this phenomenon involves the assumption that a culture of strain 59 Li of *Cl. septicum* contains two types of organisms, one type requiring uracil in the medium and the other being independent of an external supply of uracil. The two types arise from one another by mutation. An inoculum of strain 59 Li, therefore, may contain some uracil-independent organisms. If this number is large, the inoculum on

our minimal medium will grow up rapidly; if small, growth will start slowly. If there are no uracil-independent organisms the inoculum will not grow unless one or more of its members mutates. Verification of this hypothesis demands a demonstration of the two types of bacteria and their origin by mutation.

A culture of 59 Li which, after a lag period of variable length, has grown on medium devoid of uracil will contain, on this basis, at least a reasonably large number of uracil-independent bacteria. An inoculum from such a culture proved to be independent of uracil on our basal medium and upon repeated transfer showed regular growth and a short lag period. This uracil-independent strain was plated on blood-agar and thirteen single colonies were isolated. All of these new strains behaved like their parent and grew regularly and rapidly in the absence of uracil. Indeed the addition of 10  $\gamma$  per ml. of uracil to the medium on which one of these strains, 59 Li A, was grown was without effect (table 4). Therefore, the independent component of strain 59 Li has been identified.

TABLE 4

THE EFFECT OF URACIL ON THE GROWTH OF URACIL-INDEPENDENT STRAIN, 59 Li A

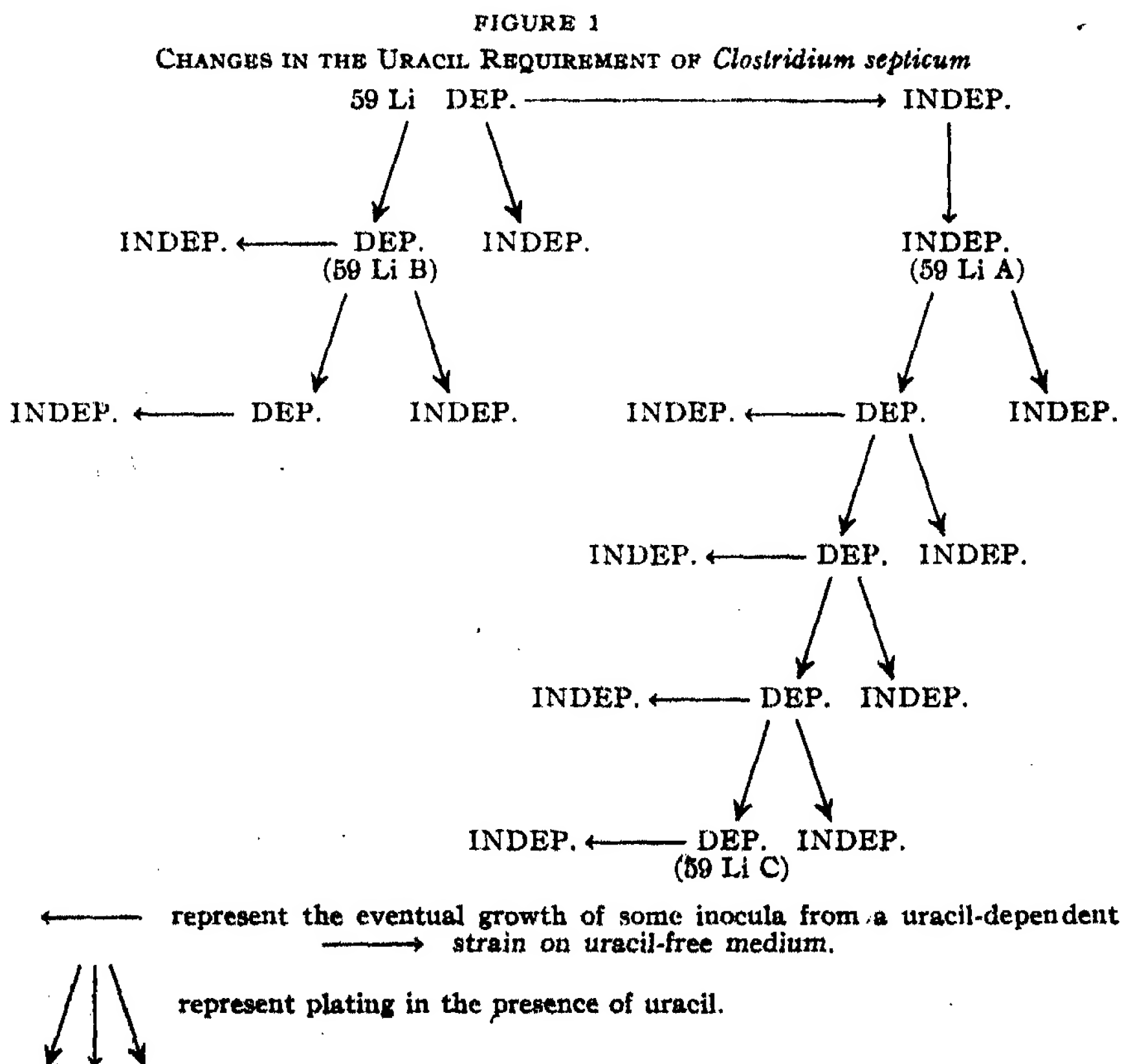
URACIL, $\gamma$ /ML.	MINUTES TO 2 MG. NITROGEN $\pm$ AVERAGE DEVIATION	GENERATION TIME, MINUTES	MG. NITROGEN AFTER 48 HOURS
0	690 $\pm$ 40	59	14.9
10	739 $\pm$ 40	62	14.5

The dependent component has also been recovered in the form of single colony isolates obtained from blood-agar plates of strain 59 Li. Of 23 such isolates 7 were dependent. These dependent strains behaved in their growth like the parent, 59 Li. Growth was erratic in the absence of uracil but regular in its presence. The fact that these dependent strains grew at all in the absence of uracil must have been due to the presence of some uracil-independent organisms. Since each of these dependent strains was isolated from a single colony their dual composition resulted from mutation of one type to the other—probably from dependence to independence. This mutation could have occurred during growth or in the spore stage in egg-meat medium, during growth of the inoculum in complete medium or while the inoculum was suspended in the uracil-free medium.<sup>9</sup> The erratic nature of the lag period could have been due to a variation in the number of independent organisms in the inoculum or to a variation in the time at which mutation occurred in the uracil-free medium.

One of the new dependent strains, 59 Li B,<sup>10</sup> was itself plated onto blood-agar. Isolation of single colonies yielded 2 strains which were uracil-dependent and 4 which were independent of uracil. Consequently, both types of cells were present in a culture not many generations from isolation as a single colony. Since care was taken to isolate well-separated colonies in single spherical zones of hemolysis on the blood-agar plates, since even

after repeated plating *all* of the dependent strains developed independence of uracil and since microscopic observation showed that agar did not cause a clumping of bacteria, it is unlikely that any colony arose from more than one organism.

Conversely when the new independent strain, 59 Li A, was plated onto blood-agar, 1 of 64 isolated strains proved to be uracil-dependent. This strain must have arisen by mutation from a uracil-independent organism and when replated gave rise to both uracil-dependent and uracil-inde-



pendent strains. This repeated plating of dependent organisms was carried out two more times with the same results, making it highly improbable that the dependent colonies arose from more than one organism. A summary of these isolations is given in figure 1.

The dual compositions of the dependent and independent strains must have resulted from the mutation of one type to the other. Thus the demands of the hypothesis have been fulfilled. The uracil-independent bacteria mutate to uracil-dependence and the uracil-dependent bacteria

mutate to a condition in which they are independent of uracil. This would account for the sudden growth which sometimes occurs in uracil-free medium after a period as long as 5 days. In an organism with a logarithmic generation time of about 80 minutes and where only about 10 generations need occur between the inoculum and final growth, 5 days seems an unreasonably long lag period on any other basis. However, we have attempted and failed to demonstrate that mutations occur while the bacteria are suspended in uracil-free medium.

The chance that a mutation will occur in a culture is a function of the number of organisms present. In a very small inoculum of dependent cells suspended in uracil-free medium, mutation, and hence growth of the culture, should be a very rare event. On the other hand in the presence of uracil such small inocula should grow regularly. Consequently, the behavior of uracil-dependent inocula of various sizes was studied in the absence and presence of uracil. When the inoculum was large and contained about  $10^6$  organisms or more (washed free of uracil by centrifuging and re-suspending in uracil-free medium three times) growth took place regularly and rapidly both in the presence and in the absence of uracil. When the inoculum contained between  $10^3$  and  $10^5$  organisms, although growth was rapid and consistent in the presence of uracil, in its absence growth was as variable as that shown in table 1. The number of organisms contained in the loop inocula which we routinely used is about  $10^3$ . When the inoculum size is as small as  $10^2$  organisms growth fails to occur not only in the absence of uracil but also in its presence. Apparently in the large inoculum there was a sufficiently large number of uracil-independent organisms to permit rapid growth. As the size of the inoculum decreases this number becomes so small that the time of onset of growth from tube to tube is variable. This variability seems to be a consequence of the number of uracil-independent cells already in the inoculum and not, at least in most cases, upon mutation in organisms suspended in the uracil-free medium.

However, there is one major difficulty with the application of this interpretation by itself—the proportion of uracil-independent organisms in a uracil-dependent inoculum is too high. A summary of the proportions of two types of organisms in dependent inocula determined by isolations from the platings described in figure 1 indicate that 68% of the cells were uracil independent (70 out of 103). In other determinations the likely average proportion of uracil-independent organisms was 77%. It may be as low as 50% or as high as 100%. In the latter case growth in uracil-free medium would be regular and rapid, an event which rarely but definitely does occur with some inocula. Although these inocula are not dependent themselves they are derived from stock cultures of spores in egg-meat medium which previously and subsequently yielded uracil-dependent inocula. In view of the high average proportion of uracil-independent organisms in



uracil-dependent cultures it is difficult to understand how inocula containing about  $10^3$  cells would vary sufficiently to account for the observed variation in the onset of growth in uracil-free medium, especially when the lag may sometimes be as long as 5 days.

Undoubtedly some other factor is involved in the variation in the onset of growth. It is probably the result of the fact that our usual inoculum of  $10^3$  cells is too close to the minimum size. The fact that there is a minimum inoculum size of more than one organism indicates that our chemically defined medium is not optimum. A certain number of cells must be present to modify the medium and permit growth. In a small inoculum where the cells are in dilute suspension there may be too slow a synthesis or too rapid a loss of some essential substance. Be that as it may, we occasionally do not provide *enough* uracil-independent cells for growth to occur. At other times the marginal number of uracil-independent organisms in the inoculum may recover and grow after a variable interval in uracil-free medium. On the other hand, in rare instances, even with an inoculum of about  $10^3$  organisms, the dependent strain will grow up rapidly in a series of tubes containing uracil-free medium, although on further test the strain will prove to be dependent. Presumably in such cases the inoculum contained close to 100% independent cells. Although the proportion of independent organisms and, perhaps, mutation to uracil-independence are involved in the variation in the onset of growth, the major factor is probably the absolute number of uracil-independent organisms in the inoculum.

Regardless of the role that mutations in uracil requirement play in the variable growth of *Cl. septicum* their properties are in themselves interesting. The organism is able to undergo a reversible change in its ability to synthesize uracil. If we assume that there is no selection in favor of either of the two types of organisms when together in the presence of uracil, we may conclude on the basis of the frequencies of the two types that mutations to uracil-dependence are much less frequent than mutations to uracil-independence. The equilibrium proportions seem to involve a preponderance of uracil-independent organisms. About 98% (63 out of 64) of the isolates from platings of 59 Li A were uracil-independent. An independent determination of the proportion was made by comparing the number of organisms from the same culture which would form colonies in the presence of uracil, with the number which formed colonies in its absence. Ten cultures were examined and again 98% of the organisms were uracil-independent. When determined by this method cultures derived from dependent strains and grown to completion contained about 77% uracil-independent cells. Isolate counts indicated the presence of about 68% independent organisms. Judging from these proportions equilibrium must nearly be attained during the growth of the inoculum to completion. The equilibrium may sometimes be achieved in the stock culture in egg-

meat medium. Some cultures when tested within a few days after they were established from isolates may prove to be dependent upon uracil. But after a week or longer they may contain a proportion of uracil-independent cells so large as to yield inocula which grow rapidly and regularly in the absence of uracil. Other stock cultures remained uracil-dependent for periods of many months on egg-meat medium where the equilibrium proportion apparently was not achieved. Frequently as many as 5 serial transfers of some uracil-dependent strains on chemically defined medium containing uracil did not result in the assumption of equilibrium. This behavior (due to mutation rates or selection) is probably a function of variability among the organisms, but it is poorly understood.

By means of a statistical procedure devised by Luria and Delbrück<sup>1</sup> it is possible to discriminate between the random and the induced nature of these mutations. This procedure is based upon the clonal mode of reproduction in bacteria. A mutation occurring early in the growth of a culture will be represented at final growth by more mutant organisms than will represent a later mutation (i.e., assuming no selection). If the mutations are "spontaneous," and have a given chance of occurring per organism per unit time throughout the growth of the culture, a great deal of variation in the proportion of mutants from culture to culture would be expected at final growth. On the other hand, if the mutations are induced and each bacterium has a given chance of responding by mutation to the testing conditions, similar proportions of mutants should be found from culture to culture. To test the applicability of these two interpretations of mutation comparisons can be made between the variation in the proportions of mutants in a series of different cultures with the variation in a series of samples from the same culture.

When this method is applied to organisms mutating to a complete requirement for some growth factor, only mutations in the presence of that growth factor can be studied, for unless the factor is supplied by the investigator or excreted into the medium during the growth of the growth-factor-independent cells the mutant organisms will not duplicate. The infrequent occurrence of uracil-dependent mutants in our cultures may have been due to spontaneous mutations in the presence of uracil although the frequency with which they occurred was too small to enable demonstration of this. On the other hand mutation from uracil-dependence to uracil-independence seems to be very rapid. Dependent cultures derived from freshly isolated colonies contain a majority of uracil-independent organisms. Nothing is known of the role of selection between the two types of cells although by itself one independent strain grew faster and farther than a dependent strain (tables 3 and 4). The study of mutation to uracil-independence must also, of course, be made on cultures grown in medium-containing uracil.

The testing medium, on the other hand, since it is to contain no uracil, must have a chemically defined composition. We have found it very difficult to secure reproducible data when colony counts were made of pour plates containing our chemically defined medium in agar.<sup>8</sup> Therefore recourse was had to the use of 0.15% semisolid agar medium in test tubes. The colonies formed in such a loose gel were easy to count and perhaps because of the depth of medium the proper anaerobic conditions were maintained to allow for consistent results at several dilutions. A uracil-dependent strain of *Cl. septicum*, 59 Li C, was diluted with medium to which uracil had been added to contain about  $10^8$  cells per 2 ml. Ten 2-ml. lots of this suspension were incubated until growth was complete. Then from each of 9 tubes a 0.1-ml. sample was taken and added to 100 ml. of medium devoid of uracil. From the tenth 2-ml. tube nine 0.1-ml. samples were removed and likewise diluted to 100 ml. From each of these dilutions 1 ml. was taken and added to 9 ml. of semisolid agar medium containing  $10^{-7}$  uracil per ml. in test tubes. From each of these 1 ml. of the mixed suspension was removed and added to another 9 ml. of semisolid agar plus uracil. This procedure was repeated until semisolid agar tubes were obtained containing dilutions between  $10^{-8}$  and  $10^{-7}$ . From the last series of tubes 1 ml. was discarded. A similar series of dilutions was made from each of the original 100-ml. dilutions in semisolid agar tubes containing medium devoid of uracil. After 24 hours' incubation the cells, which were separated by careful mixing, had formed discrete colonies which were counted. The results are shown in table 5.

There is a 0.24 probability that the distribution of proportions of uracil-independent bacteria among the 9 samples taken from the single tube was due to chance variation such as sampling error. On the other hand, there is only a 0.007 probability that the distribution of proportions among the 9 separate tubes has a similar cause. More probably the fluctuations from tube to tube reflect the distribution of mutations at different times during the growth of the culture. In this experiment it was impossible to start the growth with an inoculum of purely uracil-dependent organisms. Hence the differences from culture to culture are obscured by the uracil-independent organisms already present in the inoculum and the variation although larger than the sampling error is less than that observed in other instances.<sup>1, 2, 8</sup>

Hence, it appears as though mutations to uracil-independence occurred throughout the growth of uracil-dependent cultures in the presence of uracil (although not necessarily at random). These mutations were selected for and not induced by the absence of uracil in the testing medium. In our studies on the back-mutation of strains of *E. coli* which have been induced by radiation to require various amino acids for growth we have been able to demonstrate a similar spontaneity of mutation. In the case

of some strains of *E. coli* where there is no selection for or against the mutant organisms and where the frequency of mutations in a full grown culture is so small as to permit the preparation of inocula which contain no independent organisms, the variance from culture to culture is enormous and may be hundreds of times the mean. These studies will be reported elsewhere.

TABLE 5  
PROPORTION OF URACIL-INDEPENDENT BACTERIA IN SAMPLES FROM DIFFERENT CULTURES, AND IN SAMPLES FROM A SINGLE CULTURE

—SAMPLES FROM DIFFERENT CULTURES—				—SAMPLES FROM A SINGLE CULTURE—			
SAMPLE NUMBER	TOTAL NUMBER OF OR- GANISMS PER SAMPLE	NUMBER OF URACIL- INDE- PENDENT ORGANISMS PER SAMPLE	PERCENTAGE URACIL-IN- DEPENDENT ORGANISMS PER SAMPLE	SAMPLE NUMBER	TOTAL NUMBER OF OR- GANISMS PER SAMPLE	NUMBER OF URACIL- INDE- PENDENT ORGANISMS PER SAMPLE	PERCENTAGE URACIL-IN- DEPENDENT ORGANISMS PER SAMPLE
	$\times \frac{9}{10} \times 10^{-8}$	$\times \frac{9}{10} \times 10^{-8}$			$\times \frac{9}{10} \times 10^{-8}$	$\times \frac{9}{10} \times 10^{-8}$	
1	0.06	0.05	83	11	28	23	82
2	10	7	70	12	28	28	100
3	15	10	67	13	46	35	76
4	16	15	94	14	48	42	88
5	3	2.3	77	15	30	21	70
6	3.1	1.5	48	16	17	15	88
7	14	13	93	17	22	20	91
8	47	34	72	18	38	27	71
9	2.2	1.6	73	19	44	41	93
Average			75				84
Variance			198				106
$\chi^2$			21.10				10.12
P			0.007				0.24

The mutations in nutritional requirement in bacteria are similar to mutations of genes in sexual organisms. The inherited biochemical deficiencies involved are similar to those shown to be associated with single genes in *Neurospora* and they can be similarly induced by chemicals and radiation.<sup>12</sup> Moreover, the mutations are spontaneously reversible in the sense that the complete re-acquisition of synthetic capacity is inherited and is not induced by the absence of the required substance from the medium. The same is sometimes true of mutants of *Neurospora*.<sup>13</sup> These similarities allow the conception of biosyntheses of bacteria under the control of specific self-duplicating factors. These factors are capable of being brought to a state where, although they no longer enable the biosynthesis to be carried out, they continue to self-duplicate and can mutate to the original condition. An understanding of the mode of transmission of these hereditary units awaits the results of future research.

*Conclusion.*—The pyrimidine, uracil, is required for the growth of a strain

of *Cl. septicum*. Organisms of this strain have a given chance to back-mutate to a condition of uracil-independence in the course of their lifetime. This mutation is spontaneous in the sense that it is not induced by the absence of uracil. Uracil-independent organisms can also mutate to a condition of uracil-dependence. During the growth of this strain of *Clostridium* there is a tendency to reach an equilibrium proportion of the two types of organisms which is well in favor of the uracil-independent form. This tendency is probably due to a differential in mutation rates although selection may play a role.

In 10 ml. of liquid medium an inoculum of at least ca. 1000 cells is required for the initiation of growth. The variable time until the onset of growth of the dependent strain in the absence of uracil is mainly due to the variable number of uracil-independent organisms in the inoculum. These organisms arose by mutation from uracil-dependent cells.

\* This work was supported in part by a grant from the Josiah Macy, Jr., Foundation.

<sup>1</sup> Luria, S. E., and Delbrück, M., *Genetics*, 28, 491 (1943).

<sup>2</sup> Witkin, E. M., these PROCEEDINGS, 32, 59 (1946).

<sup>3</sup> Demerec, M., *Ibid.*, 31, 16 (1945); *Ann. Mis. Bot. Gard.*, 32, 131 (1945).

<sup>4</sup> Roepke, R. R., Libby, R. L., and Small, M., *Jour. Bact.*, 48, 401 (1944).

<sup>5</sup> Gray, C. H., and Tatum, E. L., these PROCEEDINGS, 30, 404 (1944). These authors claim that x-ray treatment was responsible for the mutant strains they obtained in *E. coli*. However, the incidence of mutations in their x-ray-treated bacteria was not significantly greater from that in the control bacteria ( $\chi^2 = 2.0$ ;  $P = 0.15$ ). In *A. melanogenum* the difference was significant ( $\chi^2 = 8.0$ ;  $P = <0.01$ ).

<sup>6</sup> Tatum, E. L., these PROCEEDINGS, 31, 215 (1945).

<sup>7</sup> Beadle, G. W., and Tatum, E. L., *Ibid.*, 27, 499 (1941).

<sup>8</sup> Ryan, F. J., Ballentine R., and Schneider, L. K., in press.

<sup>9</sup> Unless otherwise mentioned the various strains of *Cl. septicum* were maintained as spores on egg-meat medium and inocula were prepared by transferring some egg-meat into a complete (yeast extract-tryptose) broth.<sup>9</sup> Loop inocula were used. Growth was measured turbidometrically by means of a densitometer calibrated in terms of mg. of *Cl. septicum* nitrogen.

<sup>10</sup> Proof that 59 Li A and 59 Li B were actually derived from 59 Li and are not contaminants lies in a series of similar properties. All are gram-positive anaerobic rods which grow to about the same extent on our basal medium. Hence, their vitamin requirements are not more extensive than those of 59 Li. Moreover, both can utilize pantoyl lactone instead of pantothenic acid but not  $\beta$ -alanine (cf. Ryan, F. J., Ballentine, R., Stolovy, E., Corson, M. E., and Schneider, L. K., *Jour. Am. Chem. Soc.*, 67, 1857 (1945)).

<sup>11</sup> Redowitz, E., *Amer. Jour. Clin. Path., Technical Supplement*, 5, 26 (1941.)

<sup>12</sup> Tatum, E. L., *C. S. H. Symp. Quant. Biol.*, 13, in press (1946).

<sup>13</sup> Ryan, F. J., and Lederberg, J., these PROCEEDINGS, 32, 163 (1946).



## INHERITANCE OF SEX IN FUNGI

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The fungus *Hypomyces solani* f. *cucurbitae* S & H is composed of two kinds of haploid thalli, usually designated as *A* and *a*, which must be brought together to produce the perithecial stage. *A* and *a* represent allelomorphic compatibility factors which are inherited independently from the factors for sex. In defining sex in haploid thalli we would say that a female thallus is one that produces receptive structures (perithecial primordia), a male thallus is one that produces potent fertilizing (spermatial) elements, a hermaphroditic thallus one that produces both and a neuter thallus one that produces neither. Each normal thallus of this fungus is hermaphroditic, self-sterile and inter-fertile, that is,  $A \times A$  and  $a \times a$  are sterile while  $A \times a$  and  $a \times A$  are fertile. When such hermaphroditic thalli are grown to maturity and have receptive structures (perithecial primordia) then the transfer of conidia from *A* to *a* or from *a* to *A* will result in fertilization and the production of perithecia. Any living, nucleated part of the thallus can function as the male fertilizing element.

It has been shown<sup>1</sup> that this fungus frequently mutates from the hermaphrodite to the unisexual male and that the progeny from the cross hermaphrodite  $\times$  male occur in the ratio of one hermaphrodite to one male indicating that the factors for these two characters are alleles. Recently we have obtained another mutant of this fungus which is identical with the normal hermaphrodite in the production of microconidia, macroconidia, perithecial primordia and in ability to become fecundated by conidia from the opposite compatibility group, but differs in that its conidia and other parts are unable to function as male elements. This isolate then is in effect a unisexual female and when it is mated with a hermaphrodite the progeny occurs in the ratio of one female to one hermaphrodite, indicating that the factor for female, like that for the male, is also allelomorphic to the factor for hermaphrodite. Hence the factors for male and female should also be alleles and when crossed their progeny should consist of males and females in the 1:1 ratio. This however, did not occur for when 200 single-ascospore thalli from that cross were tested four sex types appeared as follows: 84 males, 64 females, 24 hermaphrodites, and 28 neuters. These neuters though they produce both macro and microconidia in abundance are unable to function either as males or females.

The above results indicate that the factors for male and female are not alleles but that they occupy loci some distance apart in homologous chromo-

somes, that crossing over between these two loci occurs and is responsible for the appearance of neuters and hermaphrodites in the progeny from the cross female  $\times$  male.

Perhaps the complicated mating behavior observed in the higher basidiomycetes and in other fungi can be accounted for by assuming an arrangement of sex factors in their chromosomes similar to that shown above.

<sup>1</sup> Hansen, H. N., and Snyder, W. C., "The Dual Phenomenon and Sex in *Hypomyces solani* f. *cucurbitae*," *Amer. Jour. Bot.*, 30, 419-422 (1943).

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DETACHMENT FREQUENCY OF ATTACHED-X CHROMOSOMES  
IN AUTOSOMAL STRUCTURAL HETEROZYGOTES OF  
*DROSOPHILA MELANOGASTER*\*

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Segregation in  $\widehat{XX}$  females of *Drosophila melanogaster* is almost exclusively  $\widehat{XX}-Y$ ,<sup>1</sup> giving as a result regular progenies consisting of matroclinous females and patroclinous males. Very rarely  $\widehat{XX}$ -chromosomes become detached by an exchange of limbs with  $Y$ .<sup>2</sup> Such detachments, when recovered, are easily detected owing to the consequent occurrence of *exceptional* offspring carrying an  $\widehat{XY}^S$ - or  $\widehat{XY}^L$ -chromosome from the mother and a normal  $X$ - or  $Y$ -chromosome from the father. Whether or not this exchange is an act of meiotic crossing-over, or crossing-over during the oögonial divisions, or possibly of both is still undecided. But since there is a tendency for the clustering of multiple occurrences of exceptional offspring in the families of certain females, it seems reasonable to conclude that the event can occur prior to meiosis.<sup>3</sup>

It has been shown that when crossing-over is reduced or inhibited in one pair of large autosomes in the female there is an attendant increase of crossing-over in the other pair of large autosomes.<sup>4</sup> This negative correlation of crossing-over between non-homologous pairs of chromosomes has also been shown to exist between the autosomes and the  $X$ -chromosomes.<sup>5</sup> Accordingly an experiment was arranged (table 1) to determine whether autosomal inversions have any pronounced effect upon the rate of detachment of  $\widehat{XX}$  by exchange with  $Y$ . Such an effect may be expected if true crossing-over is involved because the regions of  $Y$  participating in exchanges with  $\widehat{XX}$  are necessarily genetically and structurally identical with those of  $X$ .

The fact that in table 1 the females are lower in numbers than the males is accounted for by the fact that the  $\widehat{XX}$  offspring, being homozygous for five recessive genes, are less viable than the wild-type regular males. Since exceptional (detachment) males are hemizygous for the same five mutants their recovery is probably also lowered by inviability. Now inasmuch as the greatest difference in detachment rate (i.e., between A and C) is only 1.4 times the standard error of the difference, for which  $P \sim 0.085$ , it may be concluded that these experiments provide no acceptable evidence for a difference in detachment rate of  $\widehat{XX}$  in structural homozygotes and heterozygotes. Indeed the rates of exchange of  $\widehat{XX}$  with Y in these experiments are not noticeably different from those obtained by other investigators whose values for  $\widehat{XX}$ , Y; +/+; +/+ range from 0.035% to about 0.18%, with an average value of approximately 0.063%.<sup>6</sup>

TABLE 1

$y^2, w^a cv v f$ , Y FEMALES ARE USED AS MOTHERS THROUGHOUT. THE SECOND CHROMOSOME INVERTED SEQUENCE (INDICATED AS Cy) IS: *Ins(2L + 2R)Cy, al<sup>2</sup> lt<sup>3</sup> L<sup>4</sup> sp<sup>2</sup>*. THE THIRD CHROMOSOME INVERTED SEQUENCE (INDICATED AS Cx) IS: *In(3LR)Cx, D*. A NORMAL AUTOSOMAL SEQUENCE IS REPRESENTED BY "+." THE MALE PARENTS WERE CANTON-S, WILD-TYPE. THE PER CENT EXCHANGE OF  $\widehat{XX}$  WITH Y IS EQUAL TO:  $200e/2r + e$ , WHERE  $e$  EQUALS THE NUMBER OF EXCEPTIONS RECOVERED,  $r$  THE NUMBER OF REGULAR OFFSPRING

AUTOSOMAL CONSTITUTION OF MOTHER	TOTAL ♀ ♀	DETACH. ♀ ♀	TOTAL ♂ ♂	DETACH. ♂ ♂	%-EXCHANGE OF $\widehat{XX}$ WITH Y = $\sigma_p$
(A) +/+; +/+	2982	3	3,193	4	0.11 $\pm$ 0.042
(B) Cy/+; +/+	2151	2	2,407	..	0.04 $\pm$ 0.030
(C) +/+; Cx/+	2261	2	2,441	..	0.04 $\pm$ 0.029
(D) Cy/+; Cx/+	1851	2	2,282	2	0.097 $\pm$ 0.048
Totals	9245	9	10,323	6	0.077 $\pm$ 0.020

Consistent with these results are the propositions that: (1) detachment of  $\widehat{XX}$  by exchange with Y is a mitotic rather than a meiotic process, or that detachment does not take place by ordinary crossing-over, and that (2) segregation of  $\widehat{XX}$  from Y at meiosis is not dependent upon crossing-over between  $\widehat{XX}$  and Y<sup>7</sup>. The alternatives of these statements are not favored by the evidence because, on analogy with what is known generally of crossing-over in *Drosophila melanogaster*, and without the introduction of subsidiary assumptions, it would be expected that the rate of detachment of  $\widehat{XX}$  by true meiotic crossing-over with the Y would increase in correlation with the complexity of autosomal structural heterozygosity.<sup>8</sup> Hence these results are consistent with the series of cytological and genetic tests<sup>9</sup> which collectively invalidate Darlington's hypothesis<sup>10</sup> of the conjunction of X- and Y-chromosomes by reciprocal chiasmata in brachycerous diptera.

\* This work was done in the Wm. G. Kerckhoff Laboratories of the California Institute of Technology during tenure of a John Simon Guggenheim Memorial Foundation Fellowship (1945).

<sup>1</sup> Bridges, C. B., and Gabritschewsky, E., *Z.i.A.V.*, **46**, 231-247 (1928); Stern, C., *Biol. Zentrbl.*, **49**, 718-735 (1929).

<sup>2</sup> Kaufmann, B. P., these PROCEEDINGS, **19**, 830-838 (1933); Neuhaus, M., *Z.i.A.V.*, **71**, 265-275 (1936); *Genetics*, **22**, 333-339 (1937).

<sup>3</sup> Muller, H. J., and Dippel, A. L., *Brit. Jour. Exp. Biol.*, **3**, 85-122 (1926); Rhoades, M. M., *Genetics*, **16**, 375-385 (1931); Neuhaus, M., *Z.i.A.V.*, **71**, 265-275 (1936).

<sup>4</sup> The first case of such interchromosomal effects was discovered by Sturtevant, A. H., *Carn. Inst. Wash. Pub.*, **278**, 305-341 (1919). That it is a general phenomenon in *Drosophila melanogaster* was first demonstrated by the experiments of J. Schultz and H. Redfield; see *Carn. Inst. Wash. Yrbk.*, **31**, 303-307 (1932); *Ibid.*, **32**, 298-302 (1933).

<sup>5</sup> Siderow, B. N., Sokolow, N. N., and Trofimow, I. Eu., *Genetica*, **18**, 291-312 (1936); Steinberg, A. G., *Genetics*, **21**, 615-624 (1936); Steinberg, A. G., and Fraser, F. C., *Ibid.*, **29**, 83-103 (1944).

<sup>6</sup> E.g., see Anderson, E. G., *Genetics*, **10**, 403-417 (1925); Rhoades, M. M., *Ibid.*, **16**, 375-385 (1931); Schultz, J., in: Kaufmann, B. P., these PROCEEDINGS, **19**, 830-838 (1933); Neuhaus, M., *Z.i.A.V.*, **71**, 265-275 (1936); Mainx, F., *Ibid.*, **78**, 238-245 (1940).

<sup>7</sup> The general problem of segregation in  $\widehat{XX}$ , Y females and the light it throws upon the mechanism of X-Y conjunction in meiosis of the male is discussed in: Cooper, K. W., *Genetics*, **29**, page 559 (1944).

<sup>8</sup> So far as Steinberg's data<sup>5</sup> are concerned, the general increase in crossing-over of the X in autosomal heterozygotes is apparently uniform along the right end of X although no markers of the inert region itself were involved. But in the third chromosome Steinberg and Fraser<sup>5</sup> found that the regions close to the kinetochore, as compared with more distal regions, have disproportionately heightened crossing-over in heterozygotes for inversions in the other chromosomes.

<sup>9</sup> Cooper, K. W., *Genetics*, **29**, 537-568 (1944); *Ibid.*, **30**, 472-484 (1945); *Ibid.*, **31**, 181-194 (1946).

<sup>10</sup> Darlington, C. D., *Jour. Genetics*, **24**, 65-96 (1931); *Genetics*, **19**, 95-118 (1934); Koller, P. C., and Townson, T., *Proc. Roy. Soc. Edinb.*, **53**, 130-146 (1933). See footnote 7.





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## DETERMINATION OF THE SECOND HOMOLOGY AND COHOMOLOGY GROUPS OF A SPACE BY MEANS OF HOMOTOPY INVARIANTS

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1. *Introduction.*—The purpose of this note is to give a description of the 2-dimensional homology and cohomology groups of a topological<sup>1</sup> space in terms of homotopy invariants. Besides using the first and the second homotopy groups the description uses a certain new 3-dimensional invariant which will be described below.

The method utilizes the cohomology theory of abstract groups recently developed by the authors.<sup>2</sup> Given a group  $\pi$  and an additive abelian group  $G$  with  $\pi$  as (left) operators, we define the group  $C^q(\pi, G)$  of  $q$ -dimensional cochains as the group of all functions of  $q$  variables in  $\pi$  with values in  $G$ . The coboundary  $\delta f$  of a  $q$ -cochain  $f$  is defined by

$$\begin{aligned}(\delta f)(x_1, \dots, x_{q+1}) &= x_1 f(x_1, \dots, x_{q+1}) \\ &+ \sum_{i=1}^q (-1)^i f(x_1, \dots, x_i x_{i+1}, \dots, x_{q+1}) \\ &+ (-1)^{q+1} f(x_1, \dots, x_q).\end{aligned}$$

Cochains  $f$  with  $\delta f = 0$  are called *cocycles*; they form a subgroup  $Z^q(\pi, G)$  of  $C^q(\pi, G)$ . Cocycles of the form  $\delta f$ , where  $f \in C^{q-1}(\pi, G)$  are called *co-boundaries*, form a subgroup  $B^q(\pi, G)$  of  $C^q(\pi, G)$ . Since  $\delta \delta f = 0$ ,  $B^q$  is a subgroup of  $Z^q$ . The quotient group  $Z^q/B^q$  is called the  $q$ th cohomology group of  $\pi$  over  $G$  and is denoted by  $H^q(\pi, G)$ .

2. *The Invariant  $k^3$ .* Let  $X$  be an arcwise connected topological space with base point  $x_0$ . Consider the fundamental group  $\pi_1 \approx \pi_1(X, x_0)$ . For every  $w \in \pi_1$  select a path (i.e., a singular 1-simplex)  $R(w)$  representing the element  $w$ . Given two elements  $w_1, w_2 \in \pi_1$ , and given an ordered 2-simplex  $s_2$  with vertices  $v_0, v_1, v_2$ , construct a singular 2-simplex  $R(w_1, w_2): s_2 \rightarrow X$

such that the edges  $v_0v_1$ ,  $v_1v_2$  and  $v_0v_2$  are mapped according to  $R(w_1)$ ,  $R(w_2)$  and  $R(w_1w_2)$ . Now let  $w_1, w_2, w_3 \in \pi_1$ , and let  $s_3$  be a 3-simplex with ordered vertices  $v_0, v_1, v_2, v_3$ . Consider the faces  $v_1v_2v_3$ ,  $v_0v_2v_3$ ,  $v_0v_1v_3$  and  $v_0v_1v_2$  of  $s_3$  and map them into  $X$  according to the maps  $R(w_2, w_3)$ ,  $R(w_1w_2, w_3)$ ,  $R(w_1, w_2w_3)$  and  $R(w_1, w_2)$ . There results a map  $R(w_1, w_2, w_3)$  of the boundary  $B(s_3)$  of  $s_3$  into  $X$ . Regarding  $v_0$  as the base point of  $B(s_3)$ , this determines uniquely an element  $k^3(w_1, w_2, w_3)$  of the second homotopy group<sup>8</sup>  $\pi_2 = \pi_2(X, x_0)$ . We shall regard  $k^3$  as an element of the group  $C^3(\pi_1, \pi_2)$  of the 3-dimensional cochains of  $\pi_1$  with coefficients in  $\pi_2$ , with  $\pi_1$  operating on  $\pi_2$  in the usual way.

**THEOREM 1.**  $k^3$  is a cocycle. A change of the representatives  $R(w_1)$  and  $R(w_1w_2)$  alters  $k^3$  by coboundaries. Thus  $k^3$  determines uniquely a cohomology class  $k^3 \in H^3(\pi_1, \pi_2)$  which is a topological invariant of  $(X, x_0)$ . Moreover, given any cocycle  $k^3 \in Z^3(\pi_1, \pi_2)$  belonging to the class  $k^3$ , there is a choice of representatives  $R(w)$ ,  $R(w_1, w_2)$  which produce  $k^3$ .

To see that  $k^3$  is a cocycle, let  $w_1, w_2, w_3, w_4 \in \pi_1$ , and consider a 4-simplex  $s_4$  with ordered vertices  $v_0, v_1, v_2, v_3, v_4$ . It is possible to map the boundaries of faces  $v_1v_2v_3v_4$ ,  $v_0v_2v_3v_4$ ,  $v_0v_1v_3v_4$ ,  $v_0v_1v_2v_4$ ,  $v_0v_1v_2v_3$  by the maps  $R(w_2, w_3, w_4)$ ,  $R(w_1w_2, w_3, w_4)$ ,  $R(w_1, w_2w_3, w_4)$  and  $R(w_1, w_2, w_3)$ . There results a map into  $X$  of the 2-dimensional skeleton of  $s_4$ . From the definition of addition in  $\pi_2$  and of the operators it can be shown that

$$w_1k^3(w_2, w_3, w_4) - k^3(w_1w_2, w_3, w_4) + k^3(w_1, w_2w_3, w_4) - k^3(w_1, w_2, w_3w_4) + k^3(w_1, w_2, w_3) = 0;$$

i.e.,  $\delta k^3 = 0$ .

Suppose that, while keeping the representatives  $R(w)$  unchanged, we replace  $R(w_1, w_2)$  by new representatives  $R'(w_1, w_2)$ . The two maps of the 2-simplex  $s_2$  determine an element  $h(w_1, w_2)$  of  $\pi_2$ , and again from the definition of  $\pi_2$  it follows that

$$k^3(w_1, w_2, w_3) - k'^3(w_1, w_2, w_3) = w_1h(w_2, w_3) - h(w_1w_2, w_3) + h(w_1, w_2w_3) - h(w_1, w_2);$$

i.e., that  $k^3 - k'^3 = \delta h$ . Since  $h$  can be chosen arbitrarily,  $k^3$  may be altered by an arbitrary coboundary.

Finally, if the representatives  $R(w)$  are replaced by  $R'(w)$ , then, since  $R(w)$  and  $R'(w)$  are homotopic (with end-points fixed), new representatives  $R'(w_1, w_2)$  may be chosen in such a way that  $k'^3 = k^3$ .

**3. The Second Homology Groups of  $X$ .**—In terms of  $\pi_1, \pi_2$  and the new invariant  $k^3 \in H^3(\pi_1, \pi_2)$ , we can describe the 2-dimensional homology and cohomology groups of  $X$ .

Let  $k^3 \in Z^3(\pi_1, \pi_2)$  be a cocycle in the class  $k^3$ , and let  $G$  be a (topological)

abelian group selected as coefficient group for cohomologies. Consider the additive group of pairs  $(g_1, g_2)$  such that

(3.1)  $g_1 \in C^2(\pi_1, G)$ ; i.e.,  $g_1$  is a function of two variables in  $\pi_1$  with values in  $G$ ,

(3.2)  $g_2 \in \text{Hom}(\pi_2, G)$ ; i.e.,  $g_2$  is a homomorphism of  $\pi_2$  into  $G$ ,

(3.3)  $(\delta g_1)(w_1, w_2, w_3) = g_2(k^3(w_1, w_2, w_3))$ ,

(3.4)  $g_2(w\alpha) = g_2(\alpha)$  for  $w \in \pi_1, \alpha \in \pi_2$ .

Let  $H^2(\pi_1, \pi_2, k^3, G)$  be the quotient group of all these pairs  $(g_1, g_2)$  by the subgroup of pairs of the form  $(\delta h, 0)$  where  $h \in C^1(\pi_1, G)$ .

**THEOREM 2.** *The second cohomology group  $H^2(X, G)$  of  $X$  over  $G$  is isomorphic with the group  $H^2(\pi_1, \pi_2, k^3, G)$ .*

Let  $f \in Z^2(X, G)$  be a singular cocycle.  $f$  induces a homomorphism of the integral homology group  $H_2(X) \rightarrow G$ ; combined with the natural homomorphism  $\pi_2 \rightarrow H_2(X)$  this produces a homomorphism  $g_2(f): \pi_2 \rightarrow G$  which satisfies (3.4).

Given the cocycle  $k^3 \in Z^3(\pi_1, \pi_2)$ , it follows from Theorem 1 that the representatives  $R(w)$  and  $R(w_1, w_2)$  may be selected to produce  $k^3$ . The value of the cocycle  $f$  on the singular 2-simplex  $R(w_1, w_2)$  gives an element  $g_1(f)$  of  $C^2(\pi_1, G)$ . The pair  $(g_1(f), g_2(f))$  satisfies conditions (3.1)–(3.4). If  $f = \delta f'$  for a singular 1-cochain  $f'$ , then  $(g_1(f), g_2(f)) = (\delta h, 0)$  where  $h(w)$  is the value of  $f'$  on the singular 1-simplex  $R(w)$ . The correspondence  $f \rightarrow (g_1(f), g_2(f))$  thus yields a homomorphism  $H^2(X, G) \rightarrow H^2(\pi_1, \pi_2, k^3, G)$ . The proof that this is an isomorphism onto is straightforward.

The results for the homology groups follow by the application of the Pontrjagin character theory.

**4. Decomposition of the Second Cohomology Groups.**—Let  $\pi_2^\circ$  denote the subgroup of  $\pi_2$  spanned by the elements  $\alpha = w\alpha, \alpha \in \pi_2, w \in \pi_1$ .

The correspondence  $(g_1, g_2) \rightarrow g_2$  maps  $H^2(\pi_1, \pi_2, k^3, G)$  homomorphically onto a subgroup  $J$  of  $\text{Hom}(\pi_2, G)$ . The kernel of this homomorphism is the cohomology group  $H^2(\pi_1, G)$ . In the cohomology group  $H^2(X, G)$  this kernel corresponds to the subgroup  $\Lambda^2(X, G)$  consisting of those cohomology classes which annihilate all integral spherical cycles. The isomorphism  $H^2(\pi_1, G) \approx \Lambda^2(X, G)$  is known.<sup>4</sup>

The quotient group  $H^2(X, G)/\Lambda^2(X, G)$  is isomorphic with the subgroup  $J$  of  $\text{Hom}(\pi_2, G)$ . In view of (3.4) every homomorphism in  $J$  must annihilate  $\pi_2^\circ$ . A homomorphism  $g: \pi_2 \rightarrow G$  that annihilates  $\pi_2^\circ$  induces a homomorphic mapping  $g^*: H^3(\pi_1, \pi_2) \rightarrow H^3(\pi_1, G)$ . The group  $J$  consists of those homomorphisms  $g: \pi_2 \rightarrow G$  which annihilate  $\pi_2^\circ$  and for which  $g^*k^3 = 0$ .

If  $k^3 = 0$ , then  $H^2(\pi_1, G)$  is a direct summand of  $H^2(\pi_1, \pi_2, k^3, G)$ , and  $J$  consists of the annihilators of  $\pi_2^\circ$ . In this case we then have a direct sum decomposition

$$H^2(X, G) \approx H^2(\pi_1, G) + \text{Hom}(\pi_2/\pi_2^0, G).$$

This implies that in this case  $\pi_2^0$  is the kernel of the natural homomorphism of  $\pi_2$  into the 2-dimensional integral homology group of  $X$ . Comparing this with recent results of Hopf<sup>5</sup> yields numerous examples of spaces with  $k^3 \neq 0$ .

5. *Generalizations.*—1°: The construction of  $k^3$  generalizes in an obvious fashion to yield a cohomology class  $k^{n+1} \in H^{n+1}(\pi_1, \pi_n)$ , provided that  $\pi_i = 0$  for  $1 < i < n$ . Theorems 1 and 2 generalize accordingly.

2°: Assuming  $\pi_3 = 0$ , the groups  $H^3(X, G)$  may be expressed by means of  $\pi_1$ ,  $\pi_2$  and  $k^3$ . The algebraic constructions involved are quite cumbersome.

3°: If  $\pi_1$  acts as a group of operators on  $G$ , and  $H^2(X, G)$  denotes the cohomology group of  $X$  with  $G$  as local coefficients,<sup>6</sup> Theorem 2 is still valid provided that (3.4) is replaced by  $g_2(w\alpha) = wg_2(\alpha)$ .

4°: Instead of working in the space  $X$  itself, as above, one could use the universal covering space  $\tilde{X}$  of  $X$  and regard  $\pi_1$  as a group of operators on  $\tilde{X}$ . In this approach homotopy arguments are replaced by homology arguments, and some additional generality is gained. This approach will be used in the full exposition elsewhere of the results.

<sup>1</sup> Singular homologies and cohomologies are used throughout; for these concepts see Eilenberg, S., *Ann. Math.*, **45**, 407–447 (1944).

<sup>2</sup> Eilenberg, S., and MacLane, S., *Bull. Amer. Math. Soc.*, **50**, 53 (1944); *Proc. Nat. Acad. Sci. U. S. A.*, **29**, 155–158 (1943); *Ann. Math.*, **46**, 480–509 (1945); also forthcoming papers in *Ann. Math.*

<sup>3</sup> See Robbins, H., *Trans. Amer. Math. Soc.*, **49**, 319 (1941).

<sup>4</sup> Hopf, H., *Comment. Math. Helv.*, **14**, 257–309 (1942); *Ibid.*, **15**, 27–32 (1942); *Ibid.*, **17**, 39–79 (1944); see also papers in reference 2; Freudenthal, H., *Ann. Math.*, **47**, 274–316 (1946); Eckmann, B., *Comment. Math. Helv.*, **18**, 232–282 (1946).

<sup>5</sup> Hopf, H., *Comment. Math. Helv.*, **17**, 307–326 (1945).

<sup>6</sup> Steenrod, N. E., *Ann. Math.*, **44**, 610–627 (1943); Eilenberg, S., Homology Theory of Spaces with Operators I, *Trans. Amer. Math. Soc.* (in print).

## RATIONAL FUNCTIONS OF A COMPLEX VARIABLE AND RELATED POTENTIAL CURVES

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1. We shall study curves related to rational fractional functions of a complex variable  $z = x + iy$ . Our new theorems are extensions of known results concerning curves related to rational integral functions of  $z$ .

2. Curves defined by setting the real part of a polynomial (rational integral function) in  $z$  equal to zero, are well known. These were studied initially by Briot and Bouquet, and Bocher; and later were characterized completely by Kasner. These curves were called *algebraic potential curves* by Kasner in 1901. This term is used in later papers by Loria and in the German Encyclopedia. In the present article, we shall find it convenient to use the terminology: *polynomial potential or harmonic curves*.

We shall say that an algebraic curve is a *rational potential or rational harmonic curve* if it is obtained by setting equal to zero the real part of a rational fractional function of  $z$ . The class of rational harmonic curves of course includes the class of polynomial harmonic curves.

By the degree  $r$  of a rational function is meant the maximum of the two degrees of the numerator and denominator.

If the degree of the rational function of  $z$  is  $r$ , the rational harmonic curve is given by the equation:  $P(x, y) = 0$ , where  $P$  is a polynomial in  $(x, y)$  of degree  $(2r - k)$  where  $0 \leq k \leq r$ .

In general,  $P(x, y)$  does not satisfy the Laplace equation, but it does satisfy a certain partial differential equations of fourth order.

3. A rational harmonic curve of degree  $(2r - k)$  has  $k$  real asymptotes. If  $2 \leq k \leq r$ , the  $k$  real asymptotes are concurrent and make equal angles with one another. The angle between consecutive asymptotes is  $\pi/k$ . The remaining  $2(r - k)$  asymptotes are minimal. This last result remains valid even when  $k = 0$  or  $1$ ; and also the first result remains true trivially.

When  $k = r$ , this new theorem reduces to the theorem of Briot and Bouquet concerning the asymptotes of a polynomial harmonic curve.<sup>1</sup> In this case, there are no minimal asymptotes.

4. An algebraic curve is rational harmonic of degree not exceeding  $(r + s)$  where  $r > 1$  and  $s > 1$ , if and only if it passes through the  $r^2$  foci of a curve  $C_r$  of class  $r$  and the  $s^2$  foci of a curve  $C_s$  of class  $s$  such that no minimal line contains a focus of  $C_r$  and a focus of  $C_s$ .

Two analytic curves are said to be *conjugate* if they are obtained by setting the real and imaginary parts of an analytic function of  $z$  equal to zero.

A pair of algebraic curves of degree not exceeding  $(r + s)$ , are conjugate rational harmonic if and only if they intersect orthogonally in the  $r^2$  foci of a set of confocal curves  $C_r$  and in the  $s^2$  foci of another set of confocal curves  $C_s$  such that no minimal line contains a focus of a  $C_r$  and a focus of a  $C_s$ .

When  $s$  or  $r$  is zero, these characterizations of rational harmonic curves by focal properties reduce to the corresponding theorems obtained by Kasner for polynomial potential curves.<sup>2</sup>

5. Characterizations of polynomial harmonic curves have been given by Kasner by use of the concepts of apolarity and polarity. We shall discuss the apolar and polar properties of rational harmonic curves in later



work. It is remarked that although the polars of any polynomial harmonic curve are also polynomial harmonic; the corresponding result for rational potential curves is not true; that is, the polar of a rational harmonic curve is not rational harmonic in general.

6. *The degree of the Schwarzian reflection or conformal symmetry with respect to a rational harmonic curve of degree not exceeding  $(r + s)$ , where  $0 \leq s \leq r$ , is exactly  $r^2$ . The satellite of a rational harmonic curve is the same curve.*

When  $s = 0$ , this result reduces to the corresponding theorem of Kasner with respect to the polynomial potential curves.<sup>3</sup>

7. We shall say that an algebraic curve is *inverse rational* (or *inverse polynomial*) *harmonic* if it is obtained by setting equal to zero, the real part of the inverse of a rational fractional (or integral) function of  $z$ .

*All inverse rational harmonic curves are unicursal. Conversely all unicursal curves can be identified with inverse rational harmonic curves.*

A relationship between an inverse rational harmonic curve and its associated rational function of  $z$  is given by the following result.

*If the rational function of  $z$  is of degree  $r$ , the degree of the inverse rational harmonic curve is  $(2r - k)$  where  $0 \leq k \leq r$ . There are exactly  $2(r - k)$  minimal asymptotes and at most  $k$  real asymptotes.*

Of course, a pair of conjugate inverse rational harmonic curves of degree  $(2r - k)$  will intersect orthogonally in  $(2r - k)^2$  points, at most.

*All inverse polynomial harmonic curves of degree  $r$  are the special unicursal curves tangent to the line at infinity in one real point, the order of contact being  $(r - 1)$ .*

8. The only conic sections which are rational harmonic are the circles and equilateral hyperbolas. The latter are the only conics which are polynomial harmonic.

All conic sections are inverse rational harmonic. Parabolas are the only conic sections which are inverse polynomial harmonic.

If a cubic curve is rational harmonic, it is either a special circular cubic or else it is a cubic curve with three real asymptotes which are concurrent and make an angle of  $\pi/3$  with one another. The cubic curves of the latter type are the only polynomial harmonic cubic curves.

<sup>1</sup> Briot and Bouquet, *Theorie des fonctions elliptiques*, Book IV, Chapter II, p. 226. Paris-Gauthier-Villar (1875). See also Bocher, Göttingen prize memoir.

<sup>2</sup> Kasner, "On the Algebraic Potential Curves," *Bull. Amer. Math. Soc.*, 7, 392-399 (1901). Also "Some Properties of Potential Surfaces," *Ibid.*, 8, 243-248 (1902).

<sup>3</sup> Kasner, "Algebraic Curves, Symmetries and Satellites," these PROCEEDINGS, 31, 250-252 (1945). Also *La satelite conforme de una curva algebraica general*, *Revista de la Union Matematica Argentina*, 2, 77-83 (1946) (Buenos Aires).

# INTEGRAL AND RATIONAL NUMBERS IN THE NUCLEAR DOMAIN AND THEIR SIGNIFICANCE

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Using the value of the fine structure constant given by Birge<sup>1</sup> in 1941

$$hc/e^2 = 2\pi/\alpha = 860.986 \pm 0.101. \quad (1)$$

This number is so very close to an integer that it is natural to assume that

$$hc/e^2 = 861 \quad (2)$$

exactly. In this paper we shall assume that equation (2) is *true* and *significant*. Note that

$$861 = \frac{1}{2}(42 \times 41) \quad (3)$$

so that 861 is of the form  $\frac{1}{2}n(n-1)$  with  $n = 42$ . Both these numbers 861 and 42 turn up in very interesting ways in connection with nuclei and elementary particles, as will be shown in the sequel. Also  $42 = 6 \times 7$  and the number 7 also appears frequently in connection with some of these quantities. The quantities concerned are the masses and binding energies of nuclei, both in the ground state and in excited states, the magnetic moments of nuclei and the ratios of the masses of the proton and neutron to that of the electron.

The mass of the  $H^1$  atom on the physical scale is  $1.00813 \pm 0.000017$  according to Birge.<sup>1</sup> Subtracting the mass of the electron on the physical scale, the mass of the proton is

$$M_p = 1.00758 \pm 0.000017 \text{ m. u.} \quad (4)$$

Now

$$2M_p/861 = 0.00234049 \text{ m. u.} \quad (5)$$

which is the binding energy of the deuteron within the limits of experimental error.

Using as the mass of the  $H^1$  atom the value given above and as the mass of the neutron 1.00893, table 1 gives the binding energies of the nuclei up to  $He^4$  on the supposition that the constituents are protons and neutrons. The values of the atomic masses used are taken from Appendix 6 in *Applied Nuclear Physics* by Pollard and Davidson.<sup>2</sup>

TABLE 1

ISOTOPE	ATOMIC MASS	BINDING ENERGY IN M. U.	BINDING ENERGY + 0.0023405	12 X COLUMN 4	NEAREST INTEGER TO COLUMN 5
H <sup>2</sup>	2.01472	0.00234	1.000	12.00	12
H <sup>3</sup>	3.01704	0.00895	3.824	45.89	46
He <sup>3</sup>	3.01701	0.00818	3.495	41.94	42
He <sup>4</sup>	4.00388	0.03024	12.920	155.04	155

The numbers in column five of table 1 are integral within the limits of error of column three. These integers are given in column six. This table suggests that  $2M_p/861$  and  $M_p/6 \times 861$  are natural units for the binding energies of nuclei.

$$M_p/6 \times 861 = M_p/5166 = 0.000195041 \text{ m. u.} \quad (6)$$

However, someone may suggest that since the mass of the neutron,  $M_n$ , is not very different from the mass of the proton that the quantities  $2M_n/861$  and  $M_n/5166$  would serve just as well or better than the quantities above. Actual trial shows that in that case the numbers in column five would not approximate integers as closely as those in table 1.

For convenience in further discussion names must be chosen for these units.  $2M_p/861$  will be designated a *deuterium unit*, which will often be abbreviated to D. U. in writing. This shall be regarded as a unit of mass or energy as circumstances require.

$$1/12 \text{ D. U.} = M_p/5166 \quad (7)$$

shall be designated a *prout*, the abbreviation for which shall be Pr. First note that

$$M_p = 5166 \text{ Pr.} = 7 \times 738 \text{ Pr.} \quad (8)$$

Now consider the mass of the neutron.

$$\left. \begin{aligned} M_n - M_p &= 0.00135 \text{ m. u.} \\ &= 6.92 \text{ Pr.} \end{aligned} \right\} \quad (8a)$$

Within the limits of experimental error ( $M_n - M_p$ ) is 7 seven prouts and we shall assume that it is 7 *exactly* so that

$$M_n = 5173 \text{ Pr.} = 7 \times 739 \text{ Pr.} \quad (9)$$

exactly. Note the two occurrences of the number 7 in equations (8) and (9).

These results combined with the results in table 1 lead to the conclusion that the masses of all the nuclei in table 1 in the ground state are an integral number of prouts.

This suggests the working hypothesis that the masses of all stable nuclei in the ground state are an integral number of prouts (H<sup>2</sup> is of course not stable, but it fits into the integral rule nevertheless, judging by table 1).

It is now very easy to calculate the nuclear mass in prouts of all the isotopes in table 1.

The sum of the nuclear masses of  $H^1$ ,  $n$ ,  $H^2$ ,  $H^3$ ,  $He^3$  and  $He^4$  is 72,118 prouts exactly. The sum of these nuclear masses is 14.06586 m. u. Hence by division

$$1 \text{ Pr.} = 0.0001950396 \text{ m. u.} \quad (10)$$

And

$$M_p = 1.007574 \text{ m. u.} \quad (11)$$

$$M_n = 1.008940 \text{ m. u.} \quad (12)$$

These values should be good to seven significant figures.

A good nucleus on which to test our idea that all masses of stable nuclei are an integral number of prouts is that of  $O^{16}$ , since by definition its mass is exactly 16.000000 m. u. Using (11) and (12) its binding energy is 0.136504 m. u., which by equation (10) gives 699.878 prouts. There is no difficulty in identifying this as 700 prouts exactly.

With this information it is possible to calculate astoundingly accurate values of 1 prout, 1 D. U.,  $M_p$  and  $M_n$ . Using Birge's value<sup>1</sup> of the mass of the electron on the physical scale and estimating the binding energy of the electrons in  $O^{16}$  as 0.0000020 m. u., the nuclear mass of  $O^{16}$  is  $15.9956130 \pm 0.0000021$  m. u. (13)

The nuclear mass of  $O^{16}$  is 82,012 prouts. Hence by division

$$1 \text{ prout} = 0.000195039909 \pm 26 \times 10^{-12} \text{ m. u.} \quad (14)$$

$$1 \text{ D. U.} = 0.00234047891 \pm 31 \times 10^{-11} \text{ m. u.} \quad (15)$$

$$M_p = 1.00757617 \pm 13 \times 10^{-8} \text{ m. u.} \quad (16)$$

$$M_n = 1.00894145 \pm 13 \times 10^{-8} \text{ m. u.} \quad (17)$$

$$\text{Mass of } H^1 = 1.00812478 \pm 13 \times 10^{-8} \text{ m. u.} \quad (18)$$

If one uses these very accurate values to calculate the binding energies of nuclei up to  $Ne^{21}$  from the experimentally determined atomic masses and then reduces these binding energies to prouts, one finds that in all except a few cases the results are remarkably close to an integral number of prouts. We will cite only two cases. Ewald<sup>2</sup> has determined the mass of  $C^{12}$  as  $13.007581 \pm 0.000025$ . This yields a binding energy of 531.974 prouts, which is easily identified as 532 prouts. His value for  $N^{15}$  is  $15.004934 \pm 0.000030$ , which yields a binding energy of 633.046 prouts, which is very close to 633 prouts. The evidence is very strong in favor of our hypothesis, but it is too extensive to be given here. Above  $Ne^{21}$  the present atomic mass values appear to be less accurate, but it is natural

to assume that our hypothesis is valid throughout the entire range of nuclear masses.

It is interesting to note that out of a total of 18 nuclear masses which were determined in integral prouts, 6 of the values were divisible by 7. It seems to be true also that *the nuclear masses in prouts of all the most abundant isotopes are divisible by 7*, although the converse of this is not true. Using the values in equations (14), (17) and (18), the binding energy in prouts per particle was calculated for a very large number of nuclei scattered throughout the periodic table. Below  $\text{Ne}^{21}$  these values can be obtained exactly, above  $\text{Ne}^{21}$  in most cases only approximately. It seems to be true that this quantity can never exceed the integral value of 48 prouts (4 D. U.) per particle, although it comes very close to 48 in a number of cases. In fact this quantity probably lies between 47 and 48 for all stable nuclei between  $Z = 22$  and  $Z = 42$ . Between  $Z = 1$  and  $Z = 21$  it rises from 6 to a value between 47 to 48. Between  $Z = 42$  and  $Z = 84$  it drops gradually to about 42 prouts per particle. Above  $Z = 84$  it may fall below 42 prouts per particle.

The results just given together with other evidence lend some support to the idea that each "shell" of protons in the nucleus has 42 protons. For it is at  $Z = 43$  that the binding energy per particle begins to fall below the plateau value between 47 and 48.

Summing up the results so far, there is very considerable evidence for the following ideas:

1. There is a natural unit of mass and energy for the proton, neutron and all other stable nuclei, which we have designated the prout. In terms of this unit the masses of the neutron and all stable nuclei in the ground state are integral numbers.
2. The binding energies of stable nuclei in the ground state are an integral number of prouts.
3. The integers 42 and also 7, 861, 5166 and 5173 play an important rôle in "explaining" the masses of the proton, neutron and all nuclei. As mentioned before the number 42 plays an important rôle in the behavior of protons when combined into nuclei. One may therefore say that 42 and also probably 7 plays an important rôle in the basic structure of the universe.

It is perfectly clear that the masses of nuclei in excited states cannot always be an integral number of prouts, although this might be and evidently is true for some excited states. For the experiments with slow neutrons demonstrate the existence of energy levels with a spacing as small as 10 electron volts. This immediately suggests a natural generalization of the working hypotheses considered above. It is:

4. The binding energies of unstable nuclei and of nuclei in excited states are always a rational number of prouts.



5. The masses of unstable nuclei and of nuclei in excited states are always a rational number of prouts.

If these assumptions are correct then the disintegration energies in  $\alpha$ -ray emission and the energies of  $\gamma$ -rays must be a rational number of prouts. These conclusions have been tested on some of the best data for  $\alpha$ -rays and  $\gamma$ -rays and conclusions 4 and 5 are corroborated in sufficient number of cases to lend plausibility to these conclusions. Naturally the test can be satisfactory only when the denominator of the rational number mentioned is not too large when the rational number is expressed in its lowest terms, and also when the experimental data are sufficiently accurate. It can be shown that 1 prout is about 0.18163 Mev. We consider the evidence to be in favor of conclusions 4 and 5.

The magnetic moments of nuclei appear to be integral multiples of  $\mu_N/4 \times 42$ , where  $\mu_N$  is the nuclear magneton. The experimental evidence for this is quite good.

The experimental value of  $\beta \equiv M_p/m$  is compatible with the assumption that it is  $(16/15) \times 42 \times 41$ . If this is so, the mass of the electron is given by  $m = 45/16$  prouts.

The value of the pure number

$$\gamma \equiv \overline{GM_p^2}$$

is in the neighborhood of  $e^{2 \times 42}$ . All this shows the great importance of the number 42.

These results naturally suggest the idea that perhaps all intrinsic observable nuclear quantities are rational multiples of a natural unit for that quantity. The term intrinsic nuclear quantity does not include such quantities as cross-sections where interaction with an external entity is involved.

The nature of the numerical relationships stated in this paper appears to require that *certain* integers like 42 and 7 enter *somehow* into the fundamental equations of physics. With one or two exceptions to be mentioned immediately, this is the first time in the history of physics that *particular* integers or rational numbers have entered into the fundamental equations of physics. One exception is the spin of the ultimate particles in Bohr units, which is  $1/2$  for the proton, neutron and electron. The second exception, at least it can be counted as such, is the *three* dimensions of space and *one* of time. The integral and half-integral quantum numbers of quantum physics are not a case in point, because in that case the *whole set* of integers or half-integers enter into the fundamental theory and not merely certain ones. This appears to be an item of the utmost importance, because it may and probably will require a change in the *kind* of mathematical system used in formulating basic theory.

It seems improbable on the basis of these results that any theory of nuclear forces can be correct that does not at the same time account for the masses of the proton and neutron, and possibly all the other elementary particles, even mesotrons. None of the existing theories fulfil this criterion. The natural conclusion is that they are all inadequate. This does not necessarily mean that the existing theories are of no value whatsoever.

It seems altogether improbable that results such as those given in this paper could be obtained from a theory of the nucleus in which the constituent particles are held together by forces which are continuous functions of continuously varying space coördinates, *even with quantization*. It seems probable that the observed results can be obtained only from some theory in which all the quantities involved are rational or integral numbers. This seems to point to the necessity of "quantizing" the space and time coördinates. We use the word "quantization" here to mean any theory which by its very nature implies the existence of a minimum measurable distance and/or gives a discrete character to space-time. What the detailed nature of such "quantization" is remains to be seen.

Now this idea fits in quite well with the importance of the number 42. For the Riemann-Christoffel tensor of space-time has 21 *different* components. The Riemann-Christoffel tensor is one of the most fundamental tensors of a space. If we multiply 21 by 2 for some reason, possibly spin, we get the mysterious number 42. Since in the special theory of relativity the Riemann-Christoffel tensor is zero, this leads us to the idea that the explanation of nuclear forces requires a general theory of relativity, but a general theory in which space-time is "quantized." Now in Einstein's theory of general relativity it is not the Riemann-Christoffel tensor that is important, but the contracted Riemann-Christoffel tensor. This leads us to propose the idea that nuclear structure depends on the Riemann-Christoffel tensor of a quantized space-time. Thus a particle will be some kind of singularity in a quantized space-time and will have a finite size because of the quantization of space-time. Gravitation of course will also be determined by this Riemann-Christoffel tensor, and in fact in such a way that in the limit when the atomic structure of matter is neglected we will obtain the Einstein equation of gravitation.

It is hoped that our long article on this subject will appear very soon. It is our intention to continue our study of the experimental data of nuclear physics and to seek to develop a theoretical scheme along the lines indicated here.

<sup>1</sup> Birge, R. T., *Reviews of Modern Physics*, 13, 233-239 (1941).

<sup>2</sup> Pollard, E., and Davidson, W. L., *Applied Nuclear Physics*, John Wiley & Sons Inc., New York (1942).

<sup>3</sup> Ewald, Heinz, *Zeits. f. Naturforschung*, 1, 131-136 (1946).

*ON BEHRBOHM AND PINL'S LINEARIZATION OF THE  
EQUATION OF TWO-DIMENSIONAL STEADY POLYTROPIC  
FLOW OF A COMPRESSIBLE FLUID*

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The equation for the velocity potential of a compressible fluid in two-dimensional steady polytropic flow in Cartesian coördinates  $x, y$  is

$$[\lambda + (\mu - 1)\phi_x^2 + \mu\phi_y^2]\phi_{xx} - 2\phi_x\phi_y\phi_{xy} + [\lambda + (\mu - 1)\phi_y^2 + \mu\phi_x^2]\phi_{yy} = 0, \quad (1)$$

where

$$\lambda \equiv c_0^2 + \frac{\kappa - 1}{2} q_0^2, \quad \mu \equiv \frac{1 - \kappa}{2}, \quad (2)$$

$c_0$  and  $q_0$  being reference sound and flow speeds, and  $\kappa$  the polytropic exponent.<sup>1</sup> In a recent paper Behrbohm and Pinl,<sup>2</sup> observing that when  $\lambda = 1$  and  $\mu = 1$  the equation (1) reduces to that satisfied by minimal surfaces, have generalized the Minkowski support function<sup>2</sup> to achieve a "new" linearization of the equation (1). They have shown that if  $\omega(\alpha, \beta, \gamma)$  is a function homogeneous of degree 1 satisfying the equation

$$\frac{\partial^2 \omega}{\partial \alpha^2} + \frac{\partial^2 \omega}{\partial \beta^2} + \frac{1}{\lambda + (\mu - 1) \frac{\alpha^2 + \beta^2}{\gamma^2}} \frac{\partial^2 \omega}{\partial \gamma^2} = 0, \quad (3)$$

then a solution of the equation (1) may be obtained in the parametric form

$$x = \frac{\partial \omega}{\partial \alpha}, \quad y = \frac{\partial \omega}{\partial \beta}, \quad \phi(x, y) = \frac{\partial \omega}{\partial \gamma}, \quad (4)$$

provided that after all differentiations have been carried out the parameters  $\alpha, \beta, \gamma$  are connected by the relation

$$\mu(\alpha^2 + \beta^2) + \lambda\gamma^2 = (2\mu - 1)^{2\mu} \gamma^{2-2\mu}. \quad (5)$$

In the present note we proceed to show that the equation (3) is a simple alternative form of the equation obtained by the familiar linearization of the equation (1) by means of the Legendre transformation, that the equation (5) is superfluous, that hence we may set  $\gamma$  equal to 1 and obtain a physical interpretation for the variables  $\alpha$  and  $\beta$  as Cartesian components of the velocity vector, and finally that the most natural method of solution

of the equation (3) leads directly to Tschaplygin's classical solutions of the equation (1).

As our point of departure we take the linearization of the equation (1) which is obtained by the Legendre transformation: If  $f(u, v)$  satisfies the equation

$$[\lambda + (\mu - 1)v^2 + \mu u^2]f_{uu} + 2uvf_{uv} + [\lambda + (\mu - 1)u^2 + \mu v^2]f_{vv} = 0, \quad (6)$$

then a velocity potential  $\phi(x, y)$  in Cartesian coördinates may be found from the parametric equations

$$x = f_u, \quad y = f_v, \quad \phi(x, y) = f - uf_u - vf_v. \quad (7)$$

The variables  $u$  and  $v$  are the Cartesian components of the corresponding velocity vector. Suppose now we introduce new variables  $\alpha$  and  $\beta$  by the transformation

$$u = \frac{\alpha}{\gamma}, \quad v = \frac{\beta}{\gamma}, \quad (8)$$

where  $\gamma$  is a parameter. Suppose

$$\omega(\alpha, \beta, \gamma) \equiv \gamma f\left(\frac{\alpha}{\gamma}, \frac{\beta}{\gamma}\right). \quad (9)$$

Then  $\omega$  is a homogeneous function of degree 1; the equations (7) become the equations (4); also

$$\omega_{\alpha\alpha} = \frac{1}{\gamma} f_{uu}, \quad \omega_{\alpha\beta} = \frac{1}{\gamma} f_{uv}, \quad \omega_{\beta\beta} = \frac{1}{\gamma} f_{vv}. \quad (10)$$

Substituting these expressions in the equation (6) we find that

$$\left[ \lambda + (\mu - 1) \frac{\alpha^2 + \beta^2}{\gamma^2} \right] [\omega_{\alpha\alpha} + \omega_{\beta\beta}] + \frac{1}{\gamma^2} [\alpha^2 \omega_{\alpha\alpha} + 2\alpha\beta \omega_{\alpha\beta} + \beta^2 \omega_{\beta\beta}] = 0. \quad (11)$$

But the homogeneity of  $\omega(\alpha, \beta, \gamma)$  implies that

$$\alpha^2 \omega_{\alpha\alpha} + 2\alpha\beta \omega_{\alpha\beta} + \beta^2 \omega_{\beta\beta} = \gamma^2 \omega_{\gamma\gamma}. \quad (12)$$

If we substitute this formula in the equation (11) we have at once Behrbohm and Pinl's equation (3). If we regard  $\gamma$  as a given function of  $\alpha$  and  $\beta$  rather than as an independent variable, after a more extended computation we reach the same result. The interesting geometric and algebraic apparatus used by Pinl and Behrbohm in their derivation, as well as their restriction (5), is now seen to be superfluous. All our steps are reversible, so that the equations (3) and (6) are simple transforms of each other.

There are only two independent variables in our original problem, but

solutions of the system of equations (3), (12), will involve three. These variables may be left free, or they may be normalized in any convenient way. In the minimal surface problem it is customary to impose the relation  $\alpha^2 + \beta^2 + \gamma^2 = 1$  so as to make them direction cosines of the normal to the surface, but in the compressible flow problem it is more natural to put  $\gamma$  equal to 1 and thus by equations (8) to make  $\alpha$  and  $\beta$  the Cartesian components of the velocity vector.

Behrbohm and Pinl's result is in fact not a new linearization of a non-linear equation, but rather a transformation of an old one, an illustration of a general transformation principle in which the general linear differential equation of the second order in two independent variables may be reduced to a system of two linear partial differential equations of the second order in three independent variables. In the special problem we are considering here we merely make two observations. First, the degree of homogeneity of the function  $\omega(\alpha, \beta, \gamma)$  is not essential. Suppose we have some solution of the equation (11) homogeneous of any degree, say  $k$ ; if we divide it by  $\gamma^{k-1}$  it becomes homogeneous of degree 1, and remains a solution of the equation (11). The type of solution obtained is of course the same since in the end we put  $\gamma$  equal to 1. Second, the homogeneity of  $\omega(\alpha, \beta, \gamma)$  has been used in the definition (9) and in the partial differential equation (12), but only the latter is essential, since if a function  $\omega(\alpha, \beta, \gamma)$  satisfies both the equations (3) and (12), it will certainly satisfy equation (11), and hence  $\omega(x, y, 1)$  will satisfy our original equation (7). The equation (12) is satisfied not only by functions given by the formula (9), but also by functions of form

$$\omega(\alpha, \beta, \gamma) = f(\alpha\gamma, \beta\gamma), \quad (13)$$

or by a linear combination of such functions with functions homogeneous of degree 1.

Behrbohm and Pinl's paper contains a second linearization of the equation (1) of a similar type, which I conjecture also to be a simple equivalent to the equation (6), but it is sufficiently complicated that it is unlikely to be useful in the computation of flows, so we shall not consider it here.

Although nothing can be learned from this "new" linearization which cannot be discovered by the ordinary hodograph method, we may regard the equation (3) as a convenient formal device to enable us to obtain solutions of the equation (6).

It is natural to seek solutions of the equation (3) in cylindrical coordinates:

$$\omega(\alpha, \beta, \gamma) = \pi_2(\rho, \gamma)[A_2 \cos k\theta + B_2 \sin k\theta], \quad (14)$$

where

$$\rho^2 = \alpha^2 + \beta^2, \quad \tan \theta = \frac{\beta}{\alpha}. \quad (15)$$



The equation for  $\pi_k(\rho, \gamma)$  then is

$$\frac{\partial^2 \pi_k}{\partial \rho^2} + \frac{1}{\rho} \frac{\partial \pi_k}{\partial \rho} - \frac{k^2}{\rho^2} \pi_k + \frac{1}{\lambda + (\mu - 1) \frac{\rho^2}{\gamma^2}} \frac{\partial^2 \pi_k}{\partial \gamma^2} = 0. \quad (16)$$

The most general function homogeneous of degree 1 in  $\rho$  and  $\gamma$  must be of the form

$$\pi_k(\rho, \gamma) = \gamma q^k f_k(q), \quad (17)$$

where

$$q \equiv \frac{\rho}{\gamma}. \quad (18)$$

Equation (16) now yields an ordinary differential equation for  $f_k(q)$ :

$$q[\lambda + \mu q^2] \frac{d^2 f_k}{dq^2} + [(2k + 1)(\lambda + \mu q^2) - q^2] \frac{df_k}{dq} + k(k - 1)q f_k = 0, \quad (19)$$

which by the substitution

$$s \equiv -\frac{\mu}{\lambda} q^2 \quad (20)$$

reduces to the hypergeometric equation

$$s(1 - s) \frac{d^2 f_k}{ds^2} + \left[ k + 1 - \left( k + 1 - \frac{1}{2\mu} \right) s \right] \frac{df_k}{ds} - \frac{k(k - 1)}{4\mu} f_k = 0. \quad (21)$$

Accordingly the most general solution homogeneous of degree 1 of the equation (3) which can be obtained in the form (14) is given by superposition of terms of the form

$$\begin{aligned} \omega_k(\rho, \gamma) = & \gamma C_k \left( \frac{\rho}{\gamma} \right)^k F \left( \frac{k}{2} - \frac{1}{4\mu} + \frac{1}{2}l, \frac{k}{2} - \frac{1}{4\mu} - \frac{1}{2}l; k + 1; \right. \\ & \left. - \frac{\mu}{\lambda} \left( \frac{\rho}{\gamma} \right)^2 \right) + \gamma D_k \left( \frac{\rho}{\gamma} \right)^{-k} F \left( -\frac{k}{2} - \frac{1}{4\mu} + \frac{1}{2}l, -\frac{k}{2} - \frac{1}{4\mu} - \frac{1}{2}l; \right. \\ & \left. 1 - k; -\frac{\mu}{\lambda} \left( \frac{\rho}{\gamma} \right)^2 \right), \quad (22) \end{aligned}$$

where

$$l \equiv \sqrt{k^2 \left( 1 - \frac{1}{\mu} \right) + \frac{1}{4\mu^2}}, \quad (23)$$

provided  $k$  is not an integer. If  $k$  is an integer the second solution will

involve a logarithmic term. After we place the inessential variable  $\gamma$  equal to 1, the solutions derived from this potential by the aid of formula (4) becomes identical with the classical solutions of Tschaplygin.<sup>4</sup>

In deducing the preceding solution we have used the homogeneous form (9) but we could equally well have used form (13); since  $\tan \theta = (\beta\gamma)/(\alpha\gamma)$ , instead of the formula (17) we should have had

$$\pi_k(\rho, \gamma) = (\rho\gamma)^k f_k(\rho\gamma), \quad (24)$$

and in the end our result would have been the same. In both cases, of course, the  $k$ th power of the variable is introduced as a matter of convenience only.

There are other coördinate systems in which the equation (3) will separate. One variable may be split off in any system in which azimuthal planes are one of the families of coördinate surfaces, but when the inessential variable  $\gamma$  is put equal to 1 the resulting solution will of necessity reduce to Tschaplygin's solution (22). I think it likely, but have so far been unable to prove, that in fact it is impossible to separate variables in the equation (3) in any system of coördinates in which azimuthal planes are not one of the coördinate families, and hence that Tschaplygin's solutions of the hodograph equation are the only ones obtainable by separation of the variables.

<sup>1</sup> The polytropic exponent is usually denoted by  $\gamma$  in English usage, but we follow the notation of reference (2) throughout.

<sup>2</sup> Behrbohm, H., and Pinl, M., "Neue Linearisierung der Grundgleichung der eben adiabatischen kompressiblen Potentialströmung," *Z.A.M.M.*, **21**, 193-203 (1941).

<sup>3</sup> Courant, R., and Hilbert, D., *Methoden der Mathematischen Physik*, Vol. 2, Berlin, 1937, pp. 44-46.

<sup>4</sup> Tschaplygin, S., *Gas Jets*, Moscow University Scientific Memoirs, 1902, pp. 1-121, translation published as *N.A.C.A. Technical Memorandum* No. 1063, Washington, 1944; see p. 19.

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### ERRATUM

In the article, "Reverse-Mutation and Adaptation in Leucineless *Neurospora*," these PROCEEDINGS, **32**, 163 (1946), lines 17-20 on page 165 should read: "In the 25 asci and among the 158 additional spores listed in table 2 there were no recombinations. Since these numbers test for 129 chances for recombination, of which none were fulfilled, the genes involved are probably alleles."



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THE FREQUENCY OF X-RAY-INDUCED CHROMATID BREAKS  
IN *TRADESCANTIA* AS MODIFIED BY NEAR INFRARED  
RADIATION

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A modification of the frequency of x-ray-induced chromatid breaks can be achieved by varying the environmental conditions before, during or after x-radiation. All organisms, however, do not respond in like fashion to the same environmental changes. Sax and Enzmann<sup>1</sup> have shown that low temperatures (ca. 3°C.) effectively increase the frequency of x-ray-induced breaks in *Tradescantia*; high temperatures (ca. 38°C.) were shown to have the opposite effect. In *Drosophila*, temperature changes have been demonstrated to be ineffective in altering break frequencies,<sup>2, 3</sup> although earlier studies suggested that low temperatures can lead to an increase in x-ray-induced rearrangements.<sup>4, 5</sup>

The use of other types of radiation in combination with x-rays has opened a new avenue of attack on the problem of x-ray effects in that the results relative to chromosomal break frequencies do not appear to be additive. Ultra-violet radiation (2537 Å), which can, itself, induce chromatid deletions,<sup>6, 7</sup> has been shown to produce a significant decrease in x-ray-induced rearrangements in both *Drosophila*<sup>8</sup> and *Tradescantia*.<sup>9</sup> Whether this can be attributed to an effect on the nucleic acid constituents of the nucleus remains to be determined. Near infrared radiation (<20,000 Å), on the other hand, has quite the opposite effect. Ineffective in inducing breaks or rearrangements when used alone, and as detected by present cytological techniques, it considerably enhances the action of x-rays on the chromosomes of *Drosophila* if given before or during recombination of broken chromosome ends.<sup>3, 10</sup> Whether these data may be interpreted in terms of a local physical-chemical change resulting from a preferential absorption of energy by some chromosomal component, or in terms of a

more general cellular phenomenon, is as yet unknown. The present report deals with a preliminary study made of the modifying effect of near infrared radiation on x-ray-induced break and rearrangement frequencies in the microspore chromosomes of *Tradescantia*. The *Drosophila* results have been confirmed, and the investigation has been extended to other phases of the problem which are more readily studied in *Tradescantia*.

*Material and Methods.*—A clonal line of *Tradescantia paludosa*, Anders and Woodson, served as the experimental material for this study.<sup>11</sup>

The x-ray treatments were given by using a Coolidge-type tube with a tungsten target, operating at 170 kv. and 20 ma., and fitted with two filters, one of copper 0.25 mm. thick and the other of aluminum 1.06 mm. thick. At 40 cm., the distance from the target at which the inflorescences were treated, the dosage was 95 r units per minute, as measured by a Victoreen dosimeter. Only doses of one-half and one minute were used in the present study. All x-ray treatments were made by Mr. Henry Myers of the National Cancer Institute.

The method of applying the near infrared radiation was similar to that used by Kaufmann, Hollaender and Gay,<sup>10</sup> and described in greater detail by Hollaender, Kaufmann and Andrews.<sup>12</sup> The radiation from a 750-watt medium prefocus filament projection lamp was focused by means of a plano-convex glass lens through an iodine-saturated carbon tetrachloride filter 1½ cm. deep and a Corning heat-resistant glass filter No. 242. The inflorescences were placed at the bottom of a large culture tube which rotated slowly in the focus of the infrared beam. The culture tube was cooled by running tap water during the exposure of seven hours in the infrared beam. (Preliminary experiments had shown that maintaining the inflorescences in running tap water (18–19°C.) either before or after x-radiation did not significantly alter the frequency of induced breaks and rearrangements.) An interval of 5–10 minutes usually elapsed between the end of one type of radiation and the beginning of the other.

The treated anthers were smeared, fixed in 3:1 alcohol-acetic and stained with aceto-carmin 22–23 hours after x-radiation. This time was selected because Sax<sup>13</sup> has shown that during the summer months chromosome, as opposed to chromatid, breaks appear at about 27 hours after x-raying; therefore, the appearance of chromosome breaks would have indicated that the rate of cell division had been speeded up by the application of infrared radiation. No chromosome breaks of any kind were observed, however, indicating that if infrared does speed up the rate of cell division it was counteracted by the cooling effect of the running tap water, or else it was not accelerated to any detectable degree.

*The Effect of Pretreatment with Near Infrared Radiation.*—Kaufmann, Hollaender and Gay<sup>10</sup> have shown that pretreatment with near infrared radiation significantly increases the frequency of detectable x-ray-induced



chromosomal rearrangements in *Drosophila* as compared to the frequency obtained from the x-ray controls. Dominant lethals, however, which supposedly result from inviable types of breaks, appeared not to be affected under the conditions of their experiments. In *Tradescantia*, where it is possible to detect most types of viable and inviable breaks and rearrangements, it was found that all detectable types of breaks were increased when infrared radiation was given prior to x-radiation (table 1). Single and double (isochromatid) deletions were increased in frequency approximately 100% over the x-ray controls when a dosage of 47.5 r units was used, while the exchange type of rearrangement, either within or between chromosomes, revealed a considerably greater increase in frequency. This was again found to be true when the x-ray dosage was doubled (95 r). It would appear, therefore, that rearrangements between chromosomes, or between arms of the same chromosome, were increased disproportionately more than single or double deletions when infrared pretreatment was given to x-rayed microspores, but when it is considered that the frequency of this type of rearrangement is a function of the square of the dosage while the frequency of single and double deletions is a linear function of the dosage,<sup>13</sup> the disproportionality of the exchange type of rearrangements in table 1 is readily explained. These data appear to be best interpreted in terms of a generalized "sensitization" of the chromosomes to x-rays rather than in terms of a differential effect on various types of breaks and rearrangements.<sup>10</sup>

TABLE 1

FREQUENCY OF CHROMATID ABERRATIONS INDUCED BY X-RAYS AND AS MODIFIED BY PRETREATMENT WITH NEAR INFRARED RADIATION; DATA IN %

TREATMENT	SINGLE DELETIONS	DOUBLE DELETIONS	EXCHANGES	TOTAL CHROM.	TOTAL BREAKAGE
47.5 r	2.86	1.72	0.29	2094	4.87 $\pm$ 0.46
Infrared + 47.5 r	5.63	2.68	1.72	1902	10.50 $\pm$ 0.70
Infrared + 47.5 r	5.02	3.40	2.72	2352	11.13 $\pm$ 0.65
95 r	6.27	5.89	2.15	1578	14.32 $\pm$ 0.88
Infrared + 95 r	11.48	9.84	11.88	1464	33.19 $\pm$ 1.23

The data presented in table 1 do not exclude from consideration the possibility that the increased break frequencies obtained when infrared pretreatment was combined with x-radiation might be related to a general temperature change induced within the cell. Measurements were therefore made in the test tube during the infrared treatment, and a fall of 2-3°C. below room temperature was recorded, the fall being due to the cooling effect of the running tap water. This was paralleled by a similar drop in temperature in the interior of the buds as determined by means of a thermocouple designed by Dr. J. Gordon Carlson. To be doubly certain, however, that the effects of infrared radiation were not related

to a general temperature effect (as opposed to a highly localized temperature change due to selective absorption), several groups of buds were given seven hours of pretreatment at various controlled temperatures before x-radiation, and then kept at room temperature for 22 hours, at which time they were smeared, fixed and stained.

TABLE 2

FREQUENCY OF X-RAY-INDUCED CHROMATID ABERRATIONS AS MODIFIED BY PRETREATMENT AT VARIOUS TEMPERATURES FOR SEVEN HOURS; 47.5 r; DATA IN %

TREATMENT	SINGLE DELETIONS	DOUBLE DELETIONS	EXCHANGES	TOTAL CHROM.	TOTAL BREAKAGE
6°C.	1.64	3.21	0.00	1464	4.85 $\pm$ 0.56
20	2.44	3.32	0.00	1680	5.77 $\pm$ 0.57
25 (room temp)	2.61	4.02	0.60	996	7.23 $\pm$ 0.82
34	1.34	3.03	0.99	2016	5.35 $\pm$ 0.51

The data are presented in table 2. The rate of breaks and rearrangements did not approach the level obtained when infrared radiation preceded x-raying, but the data indicate that a possible rise in exchange rearrangements may follow a rise in temperature, due possibly to a more actively dividing cell. That a generalized temperature change does not account for the modification of break frequencies as shown in table 1 is, however, evident.

TABLE 3

FREQUENCY OF CHROMATID ABERRATIONS INDUCED BY X-RAYS AND AS MODIFIED BY PRETREATMENT WITH INFRARED FILTERED THROUGH 11 CM. OF WATER; DATA IN %

TREATMENT	SINGLE DELETIONS	DOUBLE DELETIONS	EXCHANGES	TOTAL CHROM.	TOTAL BREAKAGE
47.5 r	2.47	2.17	0.59	2028	5.27 $\pm$ 0.50
Infrared + 47.5 r	5.36	3.32	2.78	1866	11.47 $\pm$ 0.74

Kaufmann, Hollaender and Gay<sup>12</sup> stated that the region of effectiveness in the infrared spectrum lies in the neighborhood of 10,000 Å. This is the region most readily transmitted by the filter system which they used, and which is similar to that used in this study. However, some energy from longer wave-lengths up to 20,000 Å was transmitted. In an effort to determine the effective region more exactly, the infrared radiation was filtered through a round flask 11 cm. in diameter filled with distilled water. No other filter was used. According to Brackett,<sup>14</sup> this filter system should absorb all wave-lengths longer than 11,500 Å. The flask was water cooled to prevent it from becoming a secondary radiator. The inflorescences were irradiated as before for seven hours, and the data are contained in table 3. No significant deviations from the data in table 1 were obtained, indicating that wave-lengths longer than 11,500 Å are relatively ineffective in modifying the frequency of breaks. The filter systems which have been used have thus restricted consideration to those wave-lengths which

lie between 6000 and 11,500 Å. Further work is being carried out to determine the effectiveness of different wave-lengths within, as well as outside, this range.

*The Effect of Post-treatment with Near Infrared Radiation.*—Kaufmann, Hollaender and Gay<sup>10</sup> have shown that, in *Drosophila*, post-treatment of males with near infrared radiation led to a reduction in the frequency of detectable x-ray-induced chromosomal rearrangements, but this was subsequently interpreted as the result of a rapid maturation of sperm which were immature (and less sensitive to x-rays) when the flies were x-rayed, thus giving a heterogeneous sperm mixture at the time of copulation. Later Kaufmann<sup>3</sup> showed that the treatment of inseminated females with infrared radiation just prior to oviposition could modify the frequency of chromosomal changes in much the same manner as did pretreatment of males with infrared radiation.

TABLE 4

FREQUENCY OF CHROMATID ABERRATIONS INDUCED BY X-RAYS AND AS MODIFIED BY POST-TREATMENT WITH INFRARED RADIATION FOR SEVEN HOURS; DATA IN %

TREATMENT	SINGLE DELETIONS	DOUBLE DELETIONS	EXCHANGES	TOTAL CHROM.	TOTAL BREAKAGE
47.5 r	2.21	2.65	0.28	2898	5.15 ± 0.41
47.5 r + infrared	4.96	2.62	1.89	4656	9.47 ± 0.43

Since an interval of 22 hours existed between x-radiation and fixation of the chromosomes, there was an opportunity to alter the environmental conditions during the time when recombination is taking place. This was done with near infrared radiation, the length of treatment being seven hours. The data are given in table 4. The total frequency of chromatid changes was slightly lower than that obtained with pretreatments with infrared radiation, but an analysis of the individual types of changes indicated that the lowered frequency was due entirely to a lack of increase in the double deletions, the percentage remaining the same as that obtained from the x-ray controls. Single deletions and the exchange type of rearrangements were significantly increased, with the proportion of increase being comparable to that obtained with pretreatment of infrared radiation. From these data it may be inferred that double deletions are realized immediately on x-radiation, and hence are not subject to modification by post-treatment, whereas the type (or types) of break which gives rise to single deletions and exchange rearrangements is capable of remaining "open" for some time following x-radiation, and therefore its capacity for restitution or recombination can be readily altered by environmental conditions. These interpretations agree also with that obtained previously in *Tradescantia* when ultra-violet light, in combination with x-rays, served as the modifying influence.<sup>9</sup>

*The Permanency of the Effect of Near Infrared Radiation.*—The question

as to the permanency of the effect of infrared radiation on chromosome "sensitivity" can be readily answered merely by varying the time interval between pretreatments with infrared and x-radiation. This has been done in a very preliminary manner, but the data (table 5) are presented because they do yield some information relative to this aspect of the problem.

In this experiment a 250-watt, 120-volt reflector-drying lamp of the commercial variety was used as a source of near infrared, with the same filter system previously described being employed. Because of the greater output of transmitted energy, the time of exposure was reduced to approximately three hours. The infrared radiation was given prior to x-radiation, and the intervals of time between radiations were 16 and 21 hours, respectively. As with the other experiments, the treated anthers were smeared 22-23 hours after x-radiation.

TABLE 5

FREQUENCY OF X-RAY-INDUCED CHROMATID ABERRATIONS WHEN A DELAY EXISTS BETWEEN THE TIME OF NEAR INFRARED RADIATION AND TIME OF X-RADIATION; DATA IN %

TREATMENT	DELAY, HRS.	SINGLE DELETIONS	DOUBLE DELETIONS	EXCHANGES	TOTAL CHROM.	TOTAL BREAKAGE
47.5 r	..	1.09	1.36	0.136	1476	3.19 $\pm$ 0.46
Infrared + 47.5 r	0	4.42	3.47	1.77	1584	9.66 $\pm$ 0.74
Infrared + 47.5 r	16	4.89	3.83	1.89	1800	10.61 $\pm$ 0.73
Infrared + 47.5 r	21	4.39	3.73	0.88	1368	8.99 $\pm$ 0.77

The data obtained showed that the effect of near infrared radiation on the chromosomes was not dissipated within a short time, but rather that the "sensitization" of the chromosomes persisted for at least 21 hours, with all types of rearrangements showing a definite increase in frequency as compared with the x-ray controls. From these data it can be inferred that the effect of infrared radiation is not achieved by general temperature changes, or by a greater cell activity, but rather by a definite chemical or physical change within the cell which is reflected in the increased response of the chromosomes to x-radiation.

*Discussion.*—An intelligent discussion of the effects of near infrared radiation on the modification of the frequency of x-ray-induced chromosomal breaks and rearrangements is hindered and obscured by a lack of definite knowledge of the effects of this type of radiation on biological systems and processes. It is known that near infrared energies (1.2 electron volts for wave-lengths in the neighborhood of 10,000 Å) are insufficient to produce a dissociation of molecular structures, differing in this respect from the ionizing action of the more penetrating forms of radiation, and the photochemical action of the shorter ultra-violet. That some change, however, is produced, and that this change, reflected in an increased breakability of the chromosomes when exposed to x-radiation, is not of momentary duration but one of relative permanency, and hence

probably of a physical-chemical nature, is evident from the data presented. The data, on the other hand, yield no evidence as to whether or not the effect on the chromosomes is direct or indirect, although it is logical to assume that it is the chromosome rather than some cytoplasmic component which is altered. Since absorption of the near infrared can lead to molecular vibration and rotation, the possibility remains that macromolecular systems such as are known to be present in chromosomes may undergo some structural change, resulting in a "sensitized" structure which is more readily disrupted by x-rays.<sup>10</sup> The concept of a sensitized structure, however, does not appear to explain satisfactorily the data obtained from post-treatment experiments since one must consider here the effect on breaks, potential or otherwise, which are already available for recombination. Post-treatment effects may be due to a mechanism which alters chromosome movement, as suggested by Kaufmann,<sup>8</sup> but most certainly the fact that a delay of 21 hours between pretreatment with infrared radiation and x-radiation does not materially alter the rate of increase in break frequency as compared to that when x-radiation immediately follows infrared treatment precludes any consideration of chromosome movement as an explanation for the data obtained from the pretreatment studies. The possibility of two distinct mechanisms therefore exists.

The following hypothesis suggests itself as an explanation by which both pre- and post-treatment data may be related to a single mechanism. If both infrared and x-rays are capable of weakening the chromosome structure (this in addition to the normally realized x-ray breaks) in a manner which would not be detectable as distinct breaks, and which would not be realized unless further disturbed by the addition of the other type of radiation, then an additional increase in break frequency would be expected with both pre- and post-treatments with infrared radiation. The failure to obtain an increase in the frequency of double deletions following post-treatment is readily explained by assuming that this type of breakage and recombination is realized immediately on x-radiation, and is thus not subject to modification by subsequent treatment.

*Summary.*—Experiments are reported in which it has been shown that near infrared radiation, when combined with x-rays, significantly increases the frequency of x-ray-induced breaks and rearrangements in the microspore chromosomes of *Tradescantia*. All types of detectable alterations are increased by pretreatment, while post-treatment increases the frequency of single deletions and exchanges but does not increase the frequency of double deletions. A delay of 21 hours between pretreatment with infrared and x-rays does not appreciably decrease the effectiveness of infrared, suggesting that the change induced by infrared is of a relatively permanent nature. The nature of the effect of infrared is poorly understood at the present time.



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## REACTION AND SCATTERING CROSS-SECTIONS

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1. It was attempted, in two recent papers,<sup>1</sup> to give a formulation to the resonance theory of nuclear reactions<sup>2</sup> which is free from artificial assumptions. This theory deals with reactions in which two nuclei collide and either reparate without any change (elastic scattering) or undergo a reaction so that the pair separating after the collision is different from the colliding pair (nuclear reaction or excitation). If the energy of the system does not make the second alternative possible, we shall speak of a one alternative reaction which is, in reality, elastic scattering. If the collision can yield, instead of the original pair of nuclei, another pair or two alternative pairs, we shall speak of a two or three alternative reaction. Thus, e.g., the collision of  $\text{Be}^9$  and a neutron, which can yield either  $\text{Be}^9 + \text{neutron}$ , or  $\text{Li}^8 + \text{H}^2$ , or  $\text{He}^6 + \text{He}^4$ , or  $\text{Be}^{10} + \gamma\text{-ray}$ , will be called a four alternative reaction. The number of alternatives of the reaction which we investigate will be denoted by  $n$ . Contrary to what may seem from the above examples, this is usually not a very small number because most reactions can yield the end-products in numerous different states of excitation which are to be considered all as different alternatives. Reactions which yield more than two particles (e.g.,  $\text{H}^2 + \text{H}^1 = \text{H}^1 + \text{H}^2 + \text{neutron}$ ) are excluded from the present treatment.

One arrives at a rather definite energy dependence of the cross-sections of reactions of the above nature if one assumes, as was done in the paper

of reference 1 (a), that the wave function of the compound nucleus (i.e., the wave function in that part of configuration space where all particles are close together) is, in first approximation and apart from an energy dependent coefficient, independent of energy. On the other hand, if one admits, as was done in 1 (b), that the wave function of the compound nucleus may be a linear combination of several, or even infinitely many, energy independent functions with energy dependent coefficients, the conclusions for cross-sections, etc., become much less definite. This is quite natural since this last assumption is in reality no assumption because every wave function, and hence also the wave function of the compound nucleus, can be written as a linear combination of a definite set of functions. Any specialization contained in the second of the above papers is contained in the discussions in which assumptions are made about the order of magnitude of the constants occurring in the expressions for cross-sections, etc. The most important assumption discussed is that the distance of the resonance levels from each other is greater than their width.

However, even if one does not make any such assumptions, the results of the second paper are not vacuous but contain at least three general statements. The first two of these are:

- (i) That the elastic scattering cross-section does not vanish in general for any value of the energy, except in one alternative reactions.
- (ii) The reaction cross-section vanishes for discrete values of the energy in two alternative reactions. In three and more alternative reactions no reaction cross-section (and because of (i) no scattering cross-section) vanishes in general for any value of the energy.

The third result is more difficult to formulate and will not be taken up at this occasion. However, a proof which is independent of the formalism employed in reference 1 will be given for the above assertions which will, at the same time, generalize them inasmuch as the assumption of zero angular momentum of the colliding particles will not be employed.

2. It is well known that the total effective cross-section of a reaction, such as the transformation of a pair  $j$  into a pair  $l$ , is a sum of several partial cross-sections even if all particles which participate in the reactions have zero spin. These partial cross-sections correspond to the different angular momenta  $0, h, 2h, 3h, \dots, Lh, \dots$  of the colliding particles around their common center of mass:

$$\sigma_{jl} = \sum_L \sigma_{jl}^L. \quad (1)$$

The partial cross-sections can be expressed in terms of the matrix elements of the collision matrices  $U^L$

$$\sigma_{jl}^L = \frac{\pi}{k_j^2} (2L + 1) |U_{jl}^L - \delta_{jl}|^2. \quad (1a)$$

The collision matrices are  $n$  dimensional unitary symmetric matrices and they depend on the energy,  $k_j$  is the wave number corresponding to the relative motion of the colliding particles and  $\delta_{jl}$  is zero except in the case of elastic scattering, i.e., if  $j = l$ . Equations (1) and (1a) are valid not only if the spins of all colliding particles and reaction products are zero but also if there is no interaction between orbital motion and spin. It follows from the conservation law for angular momentum in both cases that the relative angular momentum of the reaction products is equal to the relative angular momentum of the colliding particles.

The above two rules (i) and (ii) will be shown to hold for the partial cross-sections  $\sigma_{jl}^L$  rather than the total cross-section  $\sigma_{jl}$ . Since the  $\sigma_{jl}^L$  vanish, in general, only for isolated values of the energy, the  $\sigma_{jl}$  itself will not vanish, in general at all. However, as long as the total energy of the system is low,  $\sigma_{jl}^L$  for  $L > 0$  will be very small and the vanishing of  $\sigma_{jl}^0$  will entail the vanishing of  $\sigma_{jl}$  also. Even if several  $\sigma_{jl}^L$  contribute substantially to the total cross-section, the vanishing of one of them has some observable significance because the  $\sigma_{jl}^L$  can be separated to some extent due to the different angular distributions with which they are connected.

If some of the reaction products or colliding particles have spins and if these interact with the orbital motion, the situation is even more complicated and will not be described in detail. The total cross-sections will become sums of even more terms than (1) indicates. All the partial cross-sections will be connected with matrix elements of a collision matrix in a way similar to (1a) and the rules (i) and (ii) remain valid for the partial cross-sections. However, the decomposition of the total cross-section into partial cross-sections will be even more difficult experimentally than in the above-described simple case. It will probably be possible only for low energies when only particles colliding with zero angular momentum can react with each other.

3. The rules (i) and (ii) will be demonstrated now by the method of counting the number of free parameters (a) in a general symmetric unitary matrix, (b) in a symmetric unitary matrix the  $jj$  element of which is 1, and (c) in a symmetric unitary matrix of which an off diagonal element vanishes. First we prove that:

The characteristic vectors of a symmetric unitary matrix can be assumed to be real. From  $U = U^*$  follows  $U^* = U^\dagger$  and from  $U^\dagger U = 1$  then  $U^* U = 1$ . Hence from  $Uv = \omega v$  follows  $U^* Uv = v = \omega U^* v$  or, taking the conjugate complex  $\omega^* Uv^* = v^*$ . Since for the characteristic value  $\omega$  of a unitary matrix  $\omega^* = \omega^{-1}$ , the last equation states that  $v^*$  is a characteristic vector of  $U$  and that it belongs to the same characteristic value to which  $v$  belongs. It then follows that the real vectors  $v + v^*$  and  $i(v^* - v)$ , i.e., the real and imaginary parts of  $v$ , are also characteristic vectors of  $U$  so that the characteristic vectors can all be assumed to be real.

(As a matter of fact, the derivation of the symmetric nature of  $U$  shows that this nature is a consequence of the real character of its characteristic vectors.) As a result,  $U$  can be written in the form

$$U = R'\Omega R \quad (2)$$

where  $\Omega$  is a diagonal matrix with diagonal elements of modulus 1 and  $R$  is a real orthogonal matrix. Conversely,  $R'\Omega R$  is evidently both symmetric and unitary.

The number of real parameters in  $\Omega$  is  $n$ , in  $R$  it is  $\frac{1}{2}n(n-1)$  so that  $U$  can be characterized by  $\frac{1}{2}n(n+1)$  real parameters.

Let us now consider a real symmetric matrix for which  $U_{jj} = 1$ . It then follows from the unitary condition that all  $U_{lj}$  and  $U_{jl}$  with  $l \neq j$  vanish. As a result, the number of free parameters in an  $n$  dimensional matrix  $U$  with  $U_{jj} = 1$  is the same as in a general  $n-1$  dimensional  $U$ , i.e.,  $\frac{1}{2}(n-1)n$ . One therefore has to fix  $\frac{1}{2}(n+1)n - \frac{1}{2}(n-1)n = n$  parameters to make an elastic scattering cross-section vanish. If one has only one parameter (the energy) at one's disposal, this will be possible only if  $n = 1$ , i.e., in one alternative reactions, that is, if, for reasons of energy, only elastic scattering is possible. This concludes the demonstration of (i).

When considering the case of a vanishing reaction cross-section, i.e.,  $U_{jl} = 0$  with  $j \neq l$ , we can assume, without loss of generality that  $j = 1$ ,  $l = 2$  so that  $U_{12} = 0$ .

If  $U$  is two-dimensional  $U_{12} = 0$  means that  $U$  is a unitary diagonal matrix. Since the modulus of the diagonal elements must be 1, such a  $U$  will have two free parameters. The general two-dimensional symmetric unitary matrix has three free parameters so that it will be possible, in general, to make  $U_{12}$  vanish by a suitable choice of the energy. This concludes the discussion of the first part of (ii).

A three-dimensional symmetric unitary  $U$  with  $U_{12} = 0$  has one of the two forms

$$\begin{vmatrix} \omega\omega'^* \cos \varphi & 0 & \omega \sin \varphi \\ 0 & \omega'' & 0 \\ \omega \sin \varphi & 0 & -\omega\omega' \cos \varphi \end{vmatrix} \text{ or } \begin{vmatrix} \omega'' & 0 & 0 \\ 0 & \omega\omega'^* \cos \varphi & \omega \sin \varphi \\ 0 & \omega \sin \varphi & -\omega\omega' \cos \varphi \end{vmatrix} \quad (3)$$

Since the modulus of all  $\omega$  must be 1, both forms (3) contain four real parameters. The general three-dimensional symmetric unitary  $U$  has six parameters so that it will not be possible, in general, to make  $U_{12} = 0$  by varying a single parameter. This demonstrates (ii) for three alternative reactions. (It also shows that if, for whatever reason,  $U_{12} = 0$ , either  $U_{23}$  or  $U_{13}$  must vanish also.)

If  $U$  has  $n = 4$  or more dimensions,  $U_{12} = 0$  can be expressed, by means of (2), as

$$U_{12} = \sum_j R_{j1}(\cos \varphi_j + i \sin \varphi_j)R_{j2} = 0, \quad (4)$$

where the diagonal elements of  $\Omega$  were denoted by  $\cos \varphi_j + i \sin \varphi_j$ . As is well known, the number of real parameters in  $R$ , if (4) is not fulfilled, can be counted as follows: the first column  $R_{j1}$  is an  $n$  dimensional vector of length 1, giving  $n - 1$  parameters; the second column  $R_{j2}$  is of length 1 and is orthogonal to the first column giving  $n - 2$  parameters; the third column of  $R$  must be a unit vector orthogonal to the first and the second column, giving  $n - 3$  parameters, etc. Altogether  $R$  has  $n - 1 + (n - 2) + (n - 3) + \dots + 1 = \frac{1}{2}(n - 1)n$  parameters. If (4) is fulfilled, the second column must be orthogonal to the first column and to the vectors with the components  $R_{j1} \cos \varphi_j$  and  $R_{j1} \sin \varphi_j$ . It has, therefore, if these three vectors are linearly independent, only  $n - 4$  parameters instead of the  $n - 2$  as above. Hence, the condition  $U_{12} = 0$  fixes two parameters and cannot be satisfied, in general, by varying the energy.

If only two of the vectors  $R_{j1}$ ,  $R_{j1} \cos \varphi_j$ ,  $R_{j1} \sin \varphi_j$  are independent,  $R_{j2}$  still has to be orthogonal to one more vector than if the condition  $U_{12} = 0$  is not imposed. In addition, the linear relation between the vectors  $R_{j1}$ ,  $R_{j1} \cos \varphi_j$ ,  $R_{j1} \sin \varphi_j$  gives at least one additional condition so that the number of free parameters in  $U$  with  $U_{12} = 0$  is, in this case, at least two smaller than it is in the general  $U$ . If both  $R_{j1} \cos \varphi_j$  and  $R_{j1} \sin \varphi_j$  are multiples of  $R_{j1}$ , all  $\varphi_j$  are equal which involves certainly more than two conditions. This shows that the conclusion of the preceding paragraph is valid in general and completes the demonstration of (ii).

<sup>1</sup> (a) E. P. Wigner, *Phys. Rev.*, **70**, 15 (1946); (b) to appear in the November, 1946, issue of *Phys. Rev.*

<sup>2</sup> Breit, G., and Wigner, E., *Ibid.*, **49**, 519, 642 (1936). For further literature see, e.g., reference 1 (a).

## ALGEBRAIC MATRIC GROUPS

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Let  $\mathfrak{G}$  be a multiplicative group of square matrices  $(a_{ij})$  of degree  $n \geq 1$  with elements  $a_{ij}$  contained in an algebraically closed field  $\mathbb{C}$ .  $\mathfrak{G}$  is called *algebraic* if there exists a set  $g$  of polynomials in  $\mathbb{C}[\dots, x_{ij}, \dots]$  such that: (a) for each  $(a_{ij})$  in  $\mathfrak{G}$  and each  $f(\dots, x_{ij}, \dots)$  in  $g$  we have  $f(\dots, a_{ij}, \dots) = 0$ ; (b) every non-singular matrix  $(a_{ij})$ , with elements in  $\mathbb{C}$  such that  $f(\dots, a_{ij}, \dots) = 0$  for each  $f(\dots, x_{ij}, \dots)$  in  $g$ , is in  $\mathfrak{G}$ . Such algebraic matric groups are encountered in the Picard-Vessiot theory of homogeneous linear ordinary differential equations, where they play a rôle similar to that played by finite permutation groups in the Galois theory of algebraic



equations. Any attempt to clarify and rigorize the Picard-Vessiot theory must include an adequate treatment of algebraic matrix groups, and preferably (if the Picard-Vessiot theory is to be completely algebraic) an algebraic one, independent of the theory of Lie groups. The present communication describes some results along these lines. A description of an algebraic development of the Picard-Vessiot theory is contained in the note immediately following.

If we take  $g$  as large as possible, so that it is unique, then  $\mathfrak{G}$  consists of the algebraic manifold of  $g$  (called the *underlying manifold* of  $\mathfrak{G}$ ) with a lower-dimensional algebraic manifold deleted. It turns out that the irreducible components of the underlying manifold of  $\mathfrak{G}$  are pairwise disjoint (except for singular matrices) and all have the same dimension, and the irreducible component containing the identity matrix  $\epsilon$  is the underlying manifold of a normal algebraic subgroup of  $\mathfrak{G}$  of finite index (= number of irreducible components). Definitions: this subgroup is the *component of the identity* of  $\mathfrak{G}$  (notation:  $\mathfrak{G}^\circ$ );  $\mathfrak{G}$  is *connected* if  $\mathfrak{G} = \mathfrak{G}^\circ$ ; the *dimension* of  $\mathfrak{G}$  is that of its underlying manifold.

We call  $\mathfrak{G}$  *antcompact* if  $\mathfrak{G}$  contains no matrix  $\neq \epsilon$  of finite order not divisible by the field characteristic  $p$ , and call  $\mathfrak{G}$  *quascompact* if every algebraic subgroup of  $\mathfrak{G}$  of order  $> 1$  contains such a matrix. By making joint use of the algebraic manifold properties and group properties of  $\mathfrak{G}$  we can prove: (1)  $\mathfrak{G}$  is *antcompact* if and only if each matrix of  $\mathfrak{G}$  is *reducible to special triangular form* (0's below the main diagonal, 1's on it), i.e., if and only if each matrix in  $\mathfrak{G}$  has all its characteristic roots equal to 1. (2)  $\mathfrak{G}$  is *quascompact* if and only if each matrix in  $\mathfrak{G}$  is *reducible to diagonal form*.

$\mathfrak{G}$  is called *solvable* if  $\mathfrak{G}$  has a normal chain in which all the factor groups are abelian (here "normal chain" signifies a normal chain in the usual sense plus the restriction that all members of the chain be algebraic).  $\mathfrak{G}$  is solvable if and only if its sequence of commutator subgroups terminates with the identity group (the commutator subgroup  $\mathfrak{G}'$  of  $\mathfrak{G}$  is the smallest algebraic subgroup of  $\mathfrak{G}$  containing  $\sigma\tau\sigma^{-1}\tau^{-1}$  for all  $\sigma$  and  $\tau$  in  $\mathfrak{G}$ ). Using several lemmas it is possible to prove that if  $\mathfrak{G}$  is *connected and solvable* then  $\mathfrak{G}$  is *reducible to triangular form* (0's below the main diagonal). The proof employs a double induction on the matrix degree  $n$  and the normal chain length  $r$ . For  $n = 1$  there is nothing to prove. Letting  $n > 1$  and supposing the result verified for matrices of lower degree, we can assume that  $\mathfrak{G}$  is irreducible (this requires a lemma asserting that the blocks of a reduced algebraic matrix group are themselves algebraic matrix groups, connected when the given reduced group is). For  $r = 1$  (i.e., for abelian  $\mathfrak{G}$ ) the result is an easy consequence of Schur's lemma. Letting  $r > 1$  and supposing the result verified for matrices of degree  $n$  with lower values of  $r$ , we see that the second member  $\mathfrak{G}_1$  of the normal chain for  $\mathfrak{G}$  of length

$r$  is reducible to triangular form (this requires a lemma asserting that  $\mathfrak{G}'$  is connected whenever  $\mathfrak{G}$  is, permitting the assumption that  $\mathfrak{G}_1$  is connected). Then an argument making use of the reducibility of  $\mathfrak{G}_1$  to triangular form and the abelian nature of  $\mathfrak{G}/\mathfrak{G}_1$  leads to a contradiction.

Using this theorem and the results 1 and 2 above, it can be shown that  $\mathfrak{G}$  is solvable and anticomcompact if and only if  $\mathfrak{G}$  is reducible to special triangular form, and if  $\mathfrak{G}$  is reducible to triangular form and is quasicompact then  $\mathfrak{G}$  is reducible to diagonal form.

Finally, we investigate the extent to which the concepts "solvable," "anticompact," and "quasicompact" are broadened by the introduction of apparently more inclusive definitions by means of conditions on a normal chain

$$\mathfrak{G} = \mathfrak{G}_0 \supseteq \mathfrak{G}_1 \supseteq \dots \supseteq \mathfrak{G}_{r-1} \supseteq \mathfrak{G}_r = \mathfrak{E}.$$

Using above-mentioned results concerning  $\mathfrak{G}^\circ$  and  $\mathfrak{G}'$  it is not hard to show that if every factor group  $\mathfrak{G}_{i-1}/\mathfrak{G}_i$  is abelian or finite then  $\mathfrak{G}^\circ$  is solvable.

If the definitions of anticomcompact and quasicompact are extended to factor groups in the obvious way, it can be proved (using a slight generalization of result 1 above) that: if every factor group  $\mathfrak{G}_{i-1}/\mathfrak{G}_i$  is anticomcompact or finite then  $\mathfrak{G}^\circ$  is anticomcompact; if every  $\mathfrak{G}_{i-1}/\mathfrak{G}_i$  is anticomcompact then  $\mathfrak{G}$  is, too. Analogous to the second part of this theorem is the result that if every  $\mathfrak{G}_{i-1}/\mathfrak{G}_i$  is quasicompact; then  $\mathfrak{G}$  is, too. I do not know whether the analog to the first part is true or false (for  $p = 0$  it is obviously true, as a finite group is then quasicompact; for  $p > 0$  a finite group may not be quasicompact, e.g., a group of order divisible by  $p$ ).

## THE PICARD-VESSIOT THEORY OF HOMOGENEOUS LINEAR ORDINARY DIFFERENTIAL EQUATIONS

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The Galois theory of homogeneous linear ordinary differential equations as developed by Picard and Vessiot is founded on the theory of Lie groups and on the general theory of differential equations.<sup>1</sup> Because of the loose state of the Lie theory at the time, the weakness then of the theory of differential equations with respect to its algebraic aspects, and the over-intimate connection with the analytic point of view, the Picard-Vessiot theory suffers from a certain lack of rigor, completeness and simplicity. The present communication describes an attempt to algebraize, rigorize, round out and extend the Picard-Vessiot theory. Use is made of the Ritt

theory of algebraic differential equations<sup>2</sup> (unavailable, of course, to Picard and Vessiot), and some results concerning algebraic groups of matrices developed for the purpose.<sup>3</sup>

Let  $\mathcal{F}$  be a differential field (ordinary or partial) of characteristic 0,  $\mathcal{G}$  a differential extension field thereof. A set of isomorphisms of  $\mathcal{G}$  over  $\mathcal{F}$  (i.e., isomorphisms of  $\mathcal{G}$  under which each element of  $\mathcal{F}$  is invariant) will be called *abundant* if for each differential field  $\mathcal{F}_1$  between  $\mathcal{F}$  and  $\mathcal{G}$  and each element  $\alpha$  in  $\mathcal{G} - \mathcal{F}_1$  there is an isomorphism in the set under which  $\alpha$  is not invariant but every element of  $\mathcal{F}_1$  is. By a previous paper<sup>4</sup> such sets of isomorphisms always exist.  $\mathcal{G}$  will be called a *normal* extension of  $\mathcal{F}$  if the group of all automorphisms of  $\mathcal{G}$  over  $\mathcal{F}$  is abundant.

Let  $\mathcal{G}$  be a normal extension of  $\mathcal{F}$ , and let  $\mathcal{G}$  be an abundant group of automorphisms of  $\mathcal{G}$  over  $\mathcal{F}$  (not necessarily the group of all such automorphisms). For any differential field  $\mathcal{F}_1$  between  $\mathcal{F}$  and  $\mathcal{G}$  let  $\mathcal{G}(\mathcal{F}_1)$  denote the group of all automorphisms in  $\mathcal{G}$  which leave invariant each element of  $\mathcal{F}_1$  (thus  $\mathcal{G}(\mathcal{F}) = \mathcal{G}$  and  $\mathcal{G}(\mathcal{G}) = \mathcal{E}$ , the identity group). Then it is easy to show that the mapping  $\mathcal{F}_1 \rightarrow \mathcal{G}(\mathcal{F}_1)$  is a one-to-one correspondence between the set of all differential fields between  $\mathcal{F}$  and  $\mathcal{G}$  and a certain set of subgroups of  $\mathcal{G}$ . Furthermore:  $\mathcal{G}(\mathcal{F}_1)$  is a normal subgroup of  $\mathcal{G}$  if and only if  $\sigma\mathcal{F}_1 = \mathcal{F}_1$  for every  $\sigma$  in  $\mathcal{G}$ ; when this condition is satisfied then  $\mathcal{F}_1$  is a normal extension of  $\mathcal{F}$  and  $\mathcal{G}/\mathcal{G}(\mathcal{F}_1)$  is isomorphic with an abundant group of automorphisms of  $\mathcal{F}_1$  over  $\mathcal{F}$ . I do not know, in the case in which  $\mathcal{G}$  is the group of all automorphisms of  $\mathcal{G}$  over  $\mathcal{F}$ , whether  $\mathcal{G}/\mathcal{G}(\mathcal{F}_1)$  is isomorphic with the group of all automorphisms of  $\mathcal{F}_1$  over  $\mathcal{F}$ .

Henceforth, suppose that  $\mathcal{F}$  is an ordinary differential field of characteristic 0 with an algebraically closed field of constants  $\mathcal{C}$ , and consider a homogeneous linear differential polynomial  $L(y) = y^{(n)} + p_1 y^{(n-1)} + \dots + p_n y$  (each  $p_i$  in  $\mathcal{F}$ ). If  $\eta_1, \dots, \eta_n$  are  $n$  solutions of  $L(y) = 0$ , linearly independent over  $\mathcal{C}$ , which are contained in some extension of  $\mathcal{F}$ , and if the differential field  $\mathcal{G}$  obtained by adjoining  $\eta_1, \dots, \eta_n$  to  $\mathcal{F}$  contains no constants not in  $\mathcal{C}$ , then  $\mathcal{G}$  will be called a *Picard-Vessiot* extension of  $\mathcal{F}$ . It turns out that every Picard-Vessiot extension  $\mathcal{G}$  of  $\mathcal{F}$  is normal, and the group  $\mathcal{G}$  of all automorphisms  $\sigma$  of  $\mathcal{G}$  over  $\mathcal{F}$  is (isomorphic with) an algebraic group (also denoted by  $\mathcal{G}$ ) of matrices  $(k_{ij})$ , with each  $k_{ij}$  in  $\mathcal{C}$ , such that  $\sigma\eta_j = \sum_{i=1}^n k_{ij}\eta_i$  ( $j = 1, \dots, n$ ). For the special case of Picard-Vessiot extensions it can be proved that the set of all groups  $\mathcal{G}(\mathcal{F}_1)$  with  $\mathcal{F}_1$  between  $\mathcal{F}$  and  $\mathcal{G}$  is identical with the set of all algebraic subgroups of  $\mathcal{G}$ , that when  $\mathcal{G}(\mathcal{F}_1)$  is a normal subgroup of  $\mathcal{G}$  then  $\mathcal{G}/\mathcal{G}(\mathcal{F}_1)$  is isomorphic with the group of all automorphisms of  $\mathcal{F}_1$  over  $\mathcal{F}$ , and that the *dimension* of the algebraic matrix group  $\mathcal{G}$  equals the *degree of transcendency* of  $\mathcal{G}$  over  $\mathcal{F}$ .

An element  $\alpha$  of an extension of  $\mathcal{F}$  is called an *integral* of an element  $a$  of that extension if  $\alpha' = a$ ;  $\alpha$  is called an *exponential of an integral* of  $a$  if  $\alpha' = a\alpha$ . A differential extension field  $\mathcal{H}$  of  $\mathcal{F}$  will be called *liouvillian*

if: (a) every constant in  $\mathcal{K}$  is in  $\mathcal{C}$ ; (b)  $\mathcal{C}$  is an extension of  $\mathcal{F}$  by means of integrals, exponentials of integrals, and algebraic functions, i.e., there is a monotonic sequence of differential fields  $\mathcal{F} = \mathcal{F}_0 \subseteq \mathcal{F}_1 \subseteq \dots \subseteq \mathcal{F}_r = \mathcal{K}$  such that for each  $i > 0$   $\mathcal{F}_i$  is obtained from  $\mathcal{F}_{i-1}$  by the differential field adjunction of a single element which is either an integral of an element of  $\mathcal{F}_{i-1}$ , an exponential of an integral of an element of  $\mathcal{F}_{i-1}$ , or algebraic over  $\mathcal{F}_{i-1}$ .

We shall have occasion to distinguish ten types of liouvillian extension, namely, extension by:

- (1) integral, exponentials of integrals, and algebraic functions (i.e., any liouvillian extension),
- (2) integrals and exponentials of integrals,
- (3) exponentials of integrals, and algebraic functions,
- (4) integrals and algebraic functions,
- (5) integrals and radicals,
- (6) exponentials of integrals,
- (7) integrals,
- (8) algebraic functions,
- (9) radicals,
- (10) rational functions (i.e., not a proper extension at all).

Corresponding to these ten types of liouvillian extension we consider ten types of algebraic matrix groups defined by properties of the component of the identity  $\mathcal{G}^\circ$  and of  $\mathcal{G}$  itself:

- (1)  $\mathcal{G}^\circ$  is solvable,
- (2)  $\mathcal{G}$  is solvable,
- (3)  $\mathcal{G}^\circ$  is solvable and quasicompact,
- (4)  $\mathcal{G}^\circ$  is solvable and anticomcompact,
- (5)  $\mathcal{G}$  is solvable and  $\mathcal{G}^\circ$  is anticomcompact,
- (6)  $\mathcal{G}$  is solvable and quasicompact,
- (7)  $\mathcal{G}$  is solvable and anticomcompact,
- (8)  $\mathcal{G}$  is finite,
- (9)  $\mathcal{G}$  is finite and solvable,
- (10)  $\mathcal{G} = \mathbb{C}$ .

It is now possible to state the following extension of Vessiot's big theorem on "solvability by quadratures." *If the Picard-Vessiot extension  $\mathcal{G}$  is contained in a liouvillian extension of  $\mathcal{F}$  then  $\mathcal{G}^\circ$  is solvable. Conversely, if  $\mathcal{G}^\circ$  is solvable then  $\mathcal{G}$  is a liouvillian extension of  $\mathcal{F}$ . In either case the liouvillian extension is of one of the types (1)–(10) if and only if the algebraic matrix group  $\mathcal{G}$  is of the corresponding type (1)–(10).*

<sup>1</sup> For the literature of the Picard-Vessiot theory see: Vessiot, E., *Encyclopédie des sciences mathématiques pures et appliquées*, tome II, vol. 3, fascicule 1, 58–170, esp. pp. 152–165 (1910).

<sup>2</sup> A general account of this theory as of 1938 is contained in: Ritt, J. F., *American Mathematical Society Semicentennial Publications*, II, 35–55 (1938).

<sup>3</sup> See the immediately preceding note. Familiarity with the results of that note will be assumed in the present communication.

<sup>4</sup> Kolchin, E. R., *Annals of Mathematics*, **43**, 724-729 (1942).

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## A CHARACTERIZATION OF A SIMPLE PLANE WEB

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The compact continuum  $M$  is said to be a simple web if there exist an upper semicontinuous collection  $G_1$  of mutually exclusive continua, and another such collection  $G_2$ , such that (1) each of these collections fills up  $M$ , (2) each of them is a dendron with respect to its elements and (3) if  $g_1$  is a continuum of  $G_1$  and  $g_2$  is a continuum of  $G_2$ , the common part of  $g_1$  and  $g_2$  exists and is totally disconnected.

In his thesis,<sup>1</sup> R. H. Bing has shown that in order that the compact plane continuum  $M$  should be a simple web it is necessary and sufficient that it should be a continuous curve which remains connected and locally connected on the removal of any countable point set. In the present paper<sup>2</sup> another characterization will be given.

**THEOREM 1.** *Every two points of a simple plane web are separated from each other in it either by each of uncountably many mutually exclusive arcs or by each of uncountably many mutually exclusive simple closed curves.*

*Proof.* Suppose  $M$  is a simple web in the plane  $E$ . The point set  $M$  is<sup>3</sup> a continuous curve and<sup>4</sup> the boundaries of its complementary domains are simple closed curves no two of which have more than one point in common. Bing introduces and makes extensive use of the notion of a subcontinuum of  $M$  which is maximal with respect to being the closure of a connected point set which is the sum of a countable number of points and boundaries of complementary domains of  $M$ . Let  $H$  denote the set of all such continua. Every element of  $H$  is a continuous curve every non-degenerate cyclic element of which is a simple closed curve bounding some complementary domain of  $M$  and the collection  $H$  is contracting. Let  $G$  denote the collection whose elements are the continua of  $H$  and the points of  $M$  that belong to no continuum of  $H$ . Suppose  $A$  and  $B$  are points of  $M$ .

Suppose first that  $A$  and  $B$  belong to the same continuum  $h$  of the collection  $H$ . There exists a non-degenerate cyclic element  $J$  of  $h$  such that if  $h-J$  exists no component of its closure contains both  $A$  and  $B$ . Let  $W$  denote the collection of all continua  $w$  such that  $w$  is either (1) a con-



tinuum of the collection  $G$  distinct from  $h$ , (2) a component of the closure of  $h-J$ , (3) a point of  $J$  which is not a limit point of any component of  $h-J$  or (4) a point of  $D$ , the complementary domain of  $M$  of which  $J$  is the boundary. The collection  $W$  is upper semicontinuous and there exists a reversible transformation  $T$  throwing the continua of  $W$  into the points of a spherical surface  $S$ , or into those of a plane  $S$ , and such that (1) if  $J'$  denotes the set of all continua of the collection  $W$  that intersect  $J$ ,  $T(J')$  is a circle and (2)  $T$  is reversibly continuous in the sense that the sequence  $w_1, w_2, w_3, \dots$  of continua of the collection  $W$  converges to a subcontinuum of the continuum  $w$  of  $W$  if and only if the sequence  $T(w_1), T(w_2), T(w_3), \dots$  of points of  $S$  converges to the point  $T(w)$  of  $S$ . Let  $A'$  and  $B'$  denote the continua of the collection  $W$  that contain  $A$  and  $B$ , respectively. In  $S$  the point  $T(A')$  belongs to the circle  $T(J')$ . Let  $Z$  denote the set of all circles lying on  $S$ , with center at  $T(A')$  and with radii less than the distance from  $T(A')$  to  $T(B')$ . For each circle  $z$  of the collection  $Z$ , let  $\alpha_z$  denote the arc of  $z$  which contains no point of  $T(D)$  but has, as its end-points, two points of  $T(J')$ . Let  $Q$  denote the collection of all such arcs  $\alpha_z$  for all circles  $z$  of the collection  $Z$ . Each arc of the collection  $Q$  separates  $T(A')$  from  $T(B')$  in  $S - T(D)$ . But the collection  $Q$  is uncountable and the set of all non-degenerate continua whose images under  $T$  are points of  $S$  is countable and thus all but a countable number of the arcs of  $Q$  are images under  $T$  of arcs in the plane  $E$ . But since every arc of  $Q$  separates  $T(A')$  from  $T(B')$  in  $S - T(D)$  therefore  $A$  is separated from  $B$  in  $M$  by every arc of which an arc of  $Q$  is the image under  $T$ . It follows that there are uncountably many mutually exclusive arcs each separating  $A$  from  $B$  in  $M$ .

Suppose now that no continuum of the collection  $H$  contains both  $A$  and  $B$ . In this case there exists a transformation  $T$  throwing the continua of  $G$  into the points of a sphere  $S$  and such that  $T$  is reversibly continuous in the sense described above. Let  $A'$  and  $B'$  denote the continua of  $G$  that contain  $A$  and  $B$ , respectively. On the spherical surface  $S$ ,  $T(A')$  is separated from  $T(B')$  by every circle on  $S$  with center at  $T(A)$  and radius less than the distance from  $T(A')$  to  $T(B')$ . All but a countable number of these circles are images under  $T$  of simple closed curves and every one of these simple closed curves separates  $A$  from  $B$  in  $M$ .

**THEOREM 2.** *If every two points of the compact continuum  $M$  are separated from each other in  $M$  by each of uncountably many mutually exclusive subcontinua of  $M$  but  $M$  has no cut point then  $M$  is not separated by any countable point set.*

*Proof.* Since every two points of  $M$  are separated from each other in  $M$  by a continuum,  $M$  is<sup>6</sup> a continuous curve. Suppose the points  $A$  and  $B$  are separated from each other in  $M$  by a countable point set  $K$ . Then there exists a closed non-degenerate subset  $H$  of  $K$  such that  $H$  separates

$A$  from  $B$  in  $M$  but no proper subset of  $H$  does so. Every point of  $H$  is a limit point of both  $M_A$  and  $M_B$ , the components of  $M - H$  that contain  $A$  and  $B$ , respectively. By hypothesis,  $H$  is non-degenerate. Let  $E$  and  $F$  denote two of its points. There exists a subcontinuum  $L$  of  $M$  separating  $E$  from  $F$  in  $M$  and containing no point of  $K$ . Since  $E + F + M_A$  and  $E + F + M_B$  are connected subsets of  $M$ ,  $L$  intersects both  $M_A$  and  $M_B$ . Hence  $L + M_A + M_B$  is connected. But it contains  $A$  and  $B$  and no point of  $H$ . This involves a contradiction.

**THEOREM 3.** *If every two points of the compact continuum  $M$  are separated from each other in  $M$  by each of uncountably many mutually exclusive subcontinua of  $M$  and  $M$  has no cut point then if  $e$  is a positive number there do not exist in  $M$  infinitely many mutually exclusive arcs of diameter more than  $e$  such that each of them is the sum of a countable number of continua  $t$  such that  $t$  is either a point or a subset of the boundary<sup>6</sup> of some complementary domain of  $M$ .*

*Proof.* Suppose that, for some  $e$ ,  $M$  contains infinitely many mutually exclusive arcs satisfying these conditions. Then there exists a sequence  $\alpha_1, \alpha_2, \alpha_3, \dots$  of them converging to some non-degenerate continuum  $N$ . Let  $E$  and  $F$  denote two points of  $N$ . There exists a subcontinuum  $T$  of  $M$  separating  $E$  from  $F$  in  $M$  and such that, if  $T$  intersects  $\alpha_n$ , every component of  $T \cdot \alpha_n$  is a subset of some segment of  $\alpha_n$  lying on the boundary of some complementary domain of  $M$ . There exist circles  $J_E$  and  $J_F$  with centers at  $E$  and  $F$ , respectively, and a sequence of mutually exclusive arcs  $E_1F_1, E_2F_2, \dots$  such that, for each  $n$ ,  $E_nF_n$  is, for some  $m$ , an interval of  $\alpha_m$  lying wholly without each of the circles  $J_E$  and  $J_F$ , except that  $E_n$  and  $F_n$  belong to  $J_E$  and  $J_F$ , respectively, and furthermore  $T$  intersects every arc of the sequence  $E_1F_1, E_2F_2, \dots$  and lies wholly without both  $J_E$  and  $J_F$ . There exist two integers  $i$  and  $j$ , less than or equal to 3, and arcs  $E_iE_j$  and  $F_iF_j$  lying on  $J_E$  and  $J_F$ , respectively, such that  $T$  intersects both  $I$ , the interior and  $E$ , the exterior, of  $J$ , the simple closed curve  $E_iF_i + F_iF_j + E_jF_j + E_jE_i$ . It follows that some component  $Q$  of  $T \cdot J$ , and therefore either of  $T \cdot (E_iF_i)$  or of  $T \cdot (E_jF_j)$ , contains both a limit point of  $T \cdot I$  and a limit point of  $T \cdot E$ . But  $Q$  is a subset of a segment of  $E_iF_i$  or of  $E_jF_j$  lying on the boundary of some complementary domain  $D$  of  $M$ . The domain  $D$  contains a point of  $M$ . This involves a contradiction.

**THEOREM 4.** *If every two points of the compact continuum  $M$  are separated from each other in  $M$  by each of uncountably many mutually exclusive subcontinua of  $M$  but  $M$  has no cut point then if  $K$  is a countable subset of  $M$ ,  $M - K$  is locally connected.*

*Proof.* A point set will be said to be of Class 1 if it is the sum of a countable number of continua such that each of them is either a point or a subset of the boundary of some complementary domain of  $M$ .

For each point  $P$  of  $M$  let  $M_P$  denote the point set consisting of  $P$  and

all points  $X$  of  $M$ , if there are any, such that  $X$  and  $P$  are the extremities of an arc of Class 1.

Suppose  $P$  is a point of  $M$ . Suppose  $A$  is a limit point of  $M_P$  not belonging to it. There exists a sequence  $P_1, P_2, P_3, \dots$  of distinct points of  $M_P - P$  converging to  $A$ . For each  $n$  there exists an arc  $PP_n$  of Class 1. Let  $n_1$  denote 1, let  $B_{n_1}$  denote  $P$  and let  $n_2$  denote the smallest positive  $n$  such that  $P_m$  does not belong to  $PP_{n_1}$  for any  $m$  greater than or equal to  $n$ . Let  $B_{n_2}$  denote the first point in the order from  $P_{n_1}$  to  $P$  that  $PP_{n_1}$  has in common with  $PP_{n_2}$  and let  $B_{n_1}P_{n_1}$  denote the interval of  $PP_{n_1}$  whose end-points are  $B_{n_1}$  and  $P_{n_1}$ . There exist an infinite sequence of positive integers  $n_1, n_2, n_3, \dots$ , an infinite sequence of points  $B_{n_1}, B_{n_2}, B_{n_3}, \dots$  and an infinite sequence of arcs  $B_{n_1}P_{n_1}, B_{n_2}P_{n_2}, \dots$  such that (1)  $B_{n_i}, B_{n_j}$  are as described, (2) for each  $j$ ,  $n_{j+1}$  is the smallest positive integer  $n$  such that  $P_m$  does not belong to  $PP_{n_i}$  for any  $m$  greater than or equal to  $n$ , and  $i$  less than or equal to  $j$ , (3)  $B_{n_{j+1}}$  is the first point, in the order from  $P_{n_{j+1}}$  to  $P$  on  $PP_{n_{j+1}}$  that  $PP_{n_{j+1}}$  has in common with the point set  $B_{n_1}P_{n_1} + B_{n_2}P_{n_2} + \dots + B_{n_j}P_{n_j}$  and (4)  $B_{n_{j+1}}P_{n_{j+1}}$  is the interval of  $PP_{n_{j+1}}$  whose end-points are  $B_{n_{j+1}}$  and  $P_{n_{j+1}}$ . No two of the arcs  $B_{n_1}P_{n_1}, B_{n_2}P_{n_2}, B_{n_3}P_{n_3}, \dots$  have more than one point in common. Suppose there exists a positive number  $\epsilon$  such that, for infinitely many integers  $i$ , the diameter of  $B_{n_i}P_{n_i}$  is greater than  $\epsilon$ . Then for each  $i$  there exists an interval of  $B_{n_i}P_{n_i}$  of diameter more than  $\epsilon$  and having neither  $B_{n_i}$  nor  $P_{n_i}$  as an end-point. This is contrary to Theorem 3. It follows that no arc of the sequence  $B_{n_1}P_{n_1}, B_{n_2}P_{n_2}, \dots$  intersects more than a finite number of the others. Hence there exists an infinite sequence  $Q_1, Q_2, Q_3, \dots$  such that (1) for each  $n$ ,  $Q_n$  is a finite collection of one or more arcs of the sequence  $B_{n_1}P_{n_1}, B_{n_2}P_{n_2}, \dots$ , (2)  $B_{n_1}P_{n_1}$  is the only element of  $Q_1$ , (3) each arc of  $Q_{n+1}$  intersects some arc of  $Q_n$ , but intersects no arc of  $Q_{n-1}$  if  $n > 1$ , (4) each arc of the sequence  $B_{n_1}P_{n_1}, B_{n_2}P_{n_2}, \dots$  belongs to some collection of the sequence  $Q_1, Q_2, Q_3, \dots$ . There exists<sup>7</sup> an infinite sequence  $m_1, m_2, m_3, \dots$  of distinct integers of the sequence  $n_1, n_2, n_3, \dots$  such that, for each  $j$ ,  $B_{m_j}P_{m_j}$  belongs to  $Q_j$  and intersects  $B_{m_{j+1}}P_{m_{j+1}}$ . If, for each  $j$ ,  $\alpha_j$  denotes the point  $B_{m_j}$  or the interval  $B_{m_j}B_{m_{j+1}}$  of  $B_{m_j}P_{m_j}$  according as  $B_{m_j}$  is or is not identical with  $B_{m_{j+1}}$ , then  $A + \alpha_1 + \alpha_2 + \dots$  is an arc from  $P$  to  $A$ . But it is of Class 1. Hence  $A$  belongs to  $M_P$  and  $M_P$  is closed.

With the aid of Theorem 3 and the fact that every two points of  $M_P$  are the extremities of an arc of Class 1 lying in  $M_P$ , it may be shown<sup>8</sup> that if  $A$  is a point of  $M_P$  and  $\epsilon$  is a positive number there exists a positive number  $\delta_\epsilon$  such that every point of  $M_P$  at a distance less than  $\delta_\epsilon$  from  $A$  lies together with  $A$  in an arc of Class 1 of diameter less than  $\epsilon$ . It follows that not only is  $M_P$  a continuous curve but every two points of a connected open subset of  $M_P$  are the extremities of an arc of Class 1 lying in that subset.

Suppose  $AXB$  is an arc of Class 1 lying in  $M_P$  and suppose no boundary

of a complementary domain of  $M$  contains a segment of  $AXB$  containing  $X$ . Suppose  $X$  does not separate  $A$  from  $B$  in  $M_P$ . Then, since  $M_P$  is a continuous curve, if  $D$  is the component of  $M_P - X$  that contains  $A$ ,  $D$  is an open subset of  $M_P - X$  containing  $B$  and therefore it contains an arc  $AYB$  of Class 1. The point set  $AXB + AYB$  contains a simple closed curve  $J$  of Class 1 containing a segment of  $AXB$  containing  $X$ . The curve  $J$  is not the boundary of any complementary domain of  $M$ . But it is the sum of two mutually exclusive point sets  $N_1$  and  $N_2$  such that  $N_1$  is countable and  $N_2$  is the sum of two or more segments each of which is a subset of some complementary domain of  $M$ . If  $I$  and  $E$  denote the interior and exterior of  $J$  the point sets  $M \cdot I$  and  $M \cdot E$  exist and  $N_1$  separates them from each other in  $M$ . But this is contrary to Theorem 2.

If  $A$  and  $B$  are points of  $M_P$  and the boundary of no complementary domain of  $M$  contains both of them there exists an arc  $ACB$  of Class 1 lying in  $M_P$  and there exists a cut point  $X$  of  $ACB$  belonging to no segment of  $ACB$  which is a subset of the boundary of a complementary domain of  $M$ . Furthermore if a point  $X$  of  $M_P$  is common to  $J_1$  and  $J_2$ , the boundaries of two different complementary domains of  $M$ , and  $A$  and  $B$  are points of  $J_1$  and  $J_2$ , respectively, distinct from  $X$ , and  $AX$  and  $BX$  are arcs lying on  $J_1$  and  $J_2$ , respectively, then  $AX + BX$  is an arc of Class 1 and the point  $X$  belongs to no segment of  $AXB$  which is a subset of any complementary domain of  $M$ . It follows that (1) if two points of  $M_P$  do not belong to the boundary of the same complementary domain of  $M$  they are separated from each other in  $M_P$  by some point and (2) if the boundaries of two complementary domains of  $M$  have a point of  $M_P$  in common that point separates  $M_P$ . Furthermore if the boundary of a complementary domain of  $M$  intersects  $M_P$  it is a subset of  $M_P$ . Hence every cyclic element of  $M_P$  is a simple closed curve which is the boundary of some complementary domain of  $M$ .

Let  $G$  denote the collection of all continua  $g$  such that, for some point  $P$  of  $M$ ,  $g$  is  $M_P$ . The continua of the collection  $G$  are mutually exclusive and if  $\epsilon$  is a positive number there are not more than a finite number of them of diameter more than  $\epsilon$ , and no continuum of  $G$  separates any two continua of  $G$  from each other in the plane. Furthermore  $G$  fills up  $M$  and the boundary of each complementary domain of  $M$  is a subset of some continuum of  $G$ . Hence  $G$  is upper semicontinuous and with respect to its elements regarded as points,  $G$  is topologically equivalent to the surface of a sphere. With the help of this fact and the fact that the surface of a sphere remains connected and locally connected on the removal of a countable point set, it may be shown that if  $K$  is a countable subset of  $M$  and  $H$  is the set of all elements of  $G$  that intersect  $K$  then (1)  $M - H^{*9}$  is connected and (2) it is locally connected at every one of its points not belonging to a non-degenerate continuum of  $G$ . But every point of a

continuum of  $H$  is a limit point of  $M - H^*$ . Hence,  $M - K$  is locally connected at every point of  $M - K$  belonging to no non-degenerate continuum of  $G$ . Suppose now that  $P$  is a point of  $M - K$  belonging to the non-degenerate continuum  $g$  of the collection  $G$ . If  $L$  denotes the point set  $(M - g) + P$  and  $N$  denotes  $L - K \cdot (M - g)$  then both  $L$  and  $N$  are locally connected at  $P$  and every point of  $g$  that does not belong to  $K$  is a limit point of  $N$ . Hence  $M - K$  is locally connected at  $P$ . Thus the omission of any countable point set leaves  $M$  locally connected.

The following theorem has now been established.

**THEOREM 5.** *In order that the compact non-degenerate plane continuum  $M$  with no cut point should be a continuous curve which remains both connected and locally connected on the removal of any countable point set, it is necessary and sufficient that for every two points  $X$  and  $Y$  of  $M$  there should exist uncountably many mutually exclusive continua each separating  $X$  from  $Y$  in  $M$ .*

<sup>1</sup> Bing, R. H., "Concerning Simple Plane Webs," *Trans. Am. Math. Soc.*, **60**, 133-148 (1946).

<sup>2</sup> In this paper, except where there is some indication to the contrary, it will be understood that space is a Euclidean plane  $E$ .

<sup>3</sup> Moore, R. L., "Concerning Webs in the Plane," these PROCEEDINGS, **29**, 389-393 (1943).

<sup>4</sup> Bing, R. H., *loc. cit.*

<sup>5</sup> Moore, R. L., "A Characterization of a Continuous Curve," *Fundamenta Mathematicae*, **VII**, 302-307 (1925).

<sup>6</sup> Since the continuous curve  $M$  has no cut point, therefore, by Theorem 9 of my paper "Concerning the Common Boundary of two Domains," *Ibid.*, **VI**, 203-213 (1924), the boundary of every complementary domain of  $M$  is a simple closed curve. Since  $M$  is not separated by any pair of its points, no two of these boundaries have more than one point in common. Cf. R. H. Bing, *loc. cit.*

<sup>7</sup> This may be established by an argument strictly analogous to that given to prove Theorem 78 in Chapter I, of my book "Foundations of Point Set Theory," *Am. Math. Soc. Colloquium Pub.*, Vol. XIII, New York (1932).

<sup>8</sup> This may be done with the aid of an argument having much in common with the argument given to prove Theorem 8 in Chapter II of the book cited in footnote 7.

<sup>9</sup> The notation  $H^*$  is used to denote the sum of all the point sets of the collection  $H$ .



# CYCLOTOMY AND TRINOMIAL EQUATIONS IN A FINITE FIELD

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H. H. Mitchell<sup>1</sup> considered a finite field of order  $q^e$  where  $q$  is any prime and  $q^e - 1 = ev$ . Since the group formed under multiplication by the non-zero elements of the field is cyclic we may represent all such elements as powers of one element, say  $g$ . Denote the number of solutions of the congruence

$$1 + \sigma_i \equiv \sigma_j \quad (1)$$

by  $[i, j]$  where  $\sigma_i$  denotes any element whose index is congruent to  $i$  modulo  $e$ .

Represent also the residue of index  $(-1)$ , modulo  $e$ , by  $\epsilon$ , so that if  $q$  is odd  $\epsilon \equiv 0, e/2$  according as  $v$  is even or odd, whereas if  $q = 2$ ,  $\epsilon \equiv 0$ .

He then found

$$\sum_i [i, 0] = v - 1; \quad \sum_j [i, j] = v \quad (2)$$

and also the following quadratic relations

$$\sum_i [i, j][k - i, m - i] = \sum_i [i, k][j - i, m - i], \quad (3)$$

where  $i \equiv 0, 1, 2, \dots, e - 1$ ;  $j \equiv 1, 2, \dots, e - 1 \pmod{e}$ , whereas if  $j \equiv 0, m \equiv k \pmod{e}$ , we must add  $v$  to the left-hand side of the equation, and if  $k \equiv 0, m \equiv j \pmod{e}$ , we must add  $v$  to the right-hand side.

The equation (1) may also be written in the form

$$1 + g^{i+re} = g^{j+se} \quad (4)$$

and we may note that  $[i, j]$  is the number of solutions  $g^r, g^s$ , of (4), if  $r, s = 0, 1, 2, \dots, \frac{q^e - 1}{e} - 1$ . In other papers<sup>2</sup> the writer considered the number of solutions  $g^r, g^w$ , of

$$1 + g^{f+we_1} = g^{h+we_2}. \quad (5)$$

If we denote the number of solutions of (5) by  $(f, h)$ , then we shall show in another paper that quadratic relations, of a bit different type from (3), exist involving such symbols. In the present paper we develop some formulae which we shall need in the later paper. For this purpose we shall employ some of the principles of the theory of cyclotomic fields.

Let  $p$  be an odd prime belonging to the exponent  $l$  modulo  $l$  and such

that  $p' = 1 + cl$  with  $l$  an odd prime and  $(c, l) = 1$ . Write  $\theta = \zeta\beta$ , where  $\zeta$  is a primitive  $l$ th root of unity and  $\beta$  is a primitive  $c$ th root of unity. Set

$$\psi_{a_2, b_2}(\theta) = \sum_{i, j} [i, j] \theta^{-b_2 i + (a_2 + b_2) j} \quad (6)$$

$$a_2 \not\equiv 0, b_2 \not\equiv 0, a_2 + b_2 \not\equiv 0 \pmod{cl}, i, j = 0, 1, \dots, cl - 1.$$

We have, as is known (Mitchell<sup>1</sup>),

$$\psi_{a_2, b_2}(\theta) \psi_{a_2, b_2}(\theta^{-1}) = p', \quad (7)$$

where  $[i, j]$  is defined as before with  $e$  replaced by  $cl$ ,  $\nu$  by  $l$ , and  $q$  by  $p$ . Set in (6),  $b_2 = b_1 l$ ,  $a_2 + b_2 = a_1 c$  with  $b_1 \not\equiv 0 \pmod{c}$ ,  $a_1 \not\equiv 0 \pmod{l}$  and this agrees with the restrictions  $a_2 \not\equiv 0, b_2 \not\equiv 0, a_2 + b_2 \not\equiv 0 \pmod{cl}$ . Hence (6) may be written

$$\psi_{a_1, b_1}(\theta) = \sum_{i, j} [i, j] \zeta^{-b_1 i + a_1 c j} \beta^{-b_1 i + a_1 c j}$$

or

$$\psi_{a_1, b_1}(\theta) = \sum_{i, j} [i, j] \zeta^{a_1 c j} \beta^{-b_1 i}.$$

We now write  $a$  in lieu of  $a_1 c$  and  $b$  in lieu of  $-b_1 l$  and put

$$\psi_{a, b}(\theta) = \sum_{i, j} [i, j] \zeta^{a j} \beta^{b i} \quad (8)$$

$$a \not\equiv 0 \pmod{l}, b \not\equiv 0 \pmod{c}.$$

Set  $j = r + lh$  with  $h$  in the set  $0, 1, 2, \dots, c - 1$ , and  $0 \leq r < l$ . Then the right-hand member of (8) becomes

$$\sum_r \sum_i \sum_h [i, r] \beta^{b i} \zeta^{a r}.$$

Similarly write  $i = v + ck$ ;  $0 \leq v < c$ ,  $0 \leq k < l$ . Then the above may be written as

$$\sum_r \sum_v \sum_{h=0}^{c-1} \sum_{k=0}^{l-1} [v + ck, r + lh] \beta^{b v} \zeta^{a r}.$$

By our<sup>3</sup> theorem on trinomial equations we have

$$\sum_{v=0}^{c-1} \sum_{k=0}^{l-1} [v + ck, r + lh]$$

is the number  $(v, r)$  of distinct solutions  $(g^v, g^r)$  of

$$1 + g^{v+ca} = g^{r+lb}$$

$\alpha$  in the set  $0, 1, \dots, l - 1$ , and  $\gamma$  in the set  $0, 1, \dots, c - 1$ . Hence (8) gives

$$\psi_{a,b}(\theta) = \sum_{i,j} (i, j) \beta^{bi} \zeta^{aj} \quad (9)$$

$i$  ranging over the set  $0, 1, \dots, c - 1$ , and  $j$  ranging over the set  $0, 1, \dots, l - 1$ .

This function may also be written, if  $i$  ranges over the values  $0, 1, \dots, cl - 1$  excepting  $(p^t - 1)/2$ ,

$$\sum_i \beta^{bi} \zeta^{a \text{ ind } (g^i + 1)}.$$

Hence we have the

**THEOREM I.** *If  $p$  is an odd prime belonging to the exponent  $t$  modulo  $l$  and such that  $p^t = 1 + cl$  with  $l$  an odd prime and  $(c, l) = 1$ . Write  $\theta = \zeta\beta$ , where  $\zeta$  is a primitive  $l$ th root of unity and  $\beta$  is a primitive  $o$ th root of unity. Let  $g$  be a primitive root of the finite field of order  $p^t$ , and denote by  $(v, r)$  the number of distinct solutions  $g^\alpha, g^\gamma$  of*

$$1 + g^{v+ca} = g^{r+br}$$

*$\alpha$  in the set  $0, 1, \dots, l - 1$  and  $\gamma$  in the set  $0, 1, \dots, c - 1$ . Associated with this number  $(v, r)$  is the cyclotomic function*

$$\psi_{a,b}(\theta) = \sum_{i,j} (i, j) \beta^{bi} \zeta^{aj}$$

*with  $a \not\equiv 0 \pmod{l}$ ,  $b \not\equiv 0 \pmod{c}$ ,  $i$  ranging over the set  $0, 1, \dots, c - 1$  and  $j$  ranging over the set  $0, 1, \dots, l - 1$ . Said function has the property*

$$\psi_{a,b}(\theta) \psi_{a,b}(\theta^{-1}) = p^t.$$

<sup>1</sup> Mitchell, *Ann. Math.*, 17, 165-177 (1916).

<sup>2</sup> These PROCEEDINGS, 31, 170-175; 189-194 (1945).

<sup>3</sup> *Ibid.*, 32, 51 (1946), Theorem I.

# ON SOME SPECIAL TRINOMIAL EQUATIONS IN A FINITE FIELD

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In another paper<sup>1</sup> we obtained results concerning the relations

$$au^e + bv^f + w^g = 0 \quad (1)$$

and

$$au^e + bv^f + 1 = 0, \quad (2)$$

in a finite field,  $F$ , of order  $p^n$ , designated by  $F(p^n)$ , where  $abuvw \neq 0$ ,  $a$  and  $b$  given elements of  $F$ . In the case where  $n = 1$  explicit expressions were found giving the number of solutions  $u^e, v^f, w^g$  in (1) and  $u^e, v^f$  in (2). In another paper<sup>2</sup> we considered the equation

$$c_1x_1^{a_1} + c_2x_2^{a_2} \dots + c_sx_s^{a_s} + c_{s+1} = 0, \quad (3)$$

where the  $a$ 's are integers such that  $0 < a \leq p^n - 1$ ;  $s \geq 2$ , the  $c$ 's belong to  $F$ ; and  $c_1, c_2 \dots c_s, x_1x_2 \dots x_s \neq 0$  in  $F(p^n)$ . It was there shown (Theorem I) that the number of solutions of (3) may be determined if we know the number of solutions of

$$ky^m + lz^m + 1 = 0, \quad (4)$$

for any given  $k$  and  $l$  in  $F(p^n)$  where  $(p^n - 1, a_i) = d_i$ ;  $i = 1, 2, \dots, s$ ; and  $m$  is the L.C.M. of  $d_1, d_2, \dots, d_s$ , provided we have a table of indices for the elements of the field under multiplication. As (1) and (2) are special cases of (3) the method of the second paper applies to each of the former. But the use of the latter method, convenient as it appears, gives us no information in some cases concerning the number of solutions of (1) or (2). This is emphatically brought out by the following investigation. In (1) set  $f = g$  and we obtain

$$au^e + bv^f + w^f = 0. \quad (5)$$

further set  $ef = p^n - 1$  with  $(e, f) = 1$ . This is the special case of Theorem I of the first paper herein mentioned, with  $f = g$ ,  $ef = p^n - 1$  and  $(e, f) = 1$ . If we apply the method of the second paper to (5) we note that since the least common multiple of  $e$  and  $f$  is  $p^n - 1$  then the equation (4) reduces to  $k + l + 1 = 0$ , and this means that we have to know what various equations of this type exist for classes of values  $k$  and  $l$ , which reduces to the problem we started with. However, the use of another method determines the number exactly as we shall now show.

Consider

$$1 + bx^f \quad (6)$$

where  $x^f$  ranges over all  $e$  possible values in the field. Each value, unless it is zero in the field, may be expressed in the form:

$$-a\rho^{es}\rho^{ft},$$

since the multiplicative cyclic group of the non-zero elements of the finite field  $F(p^n)$ , generated by  $\rho$ , can be expressed as the product of two cyclic groups of orders  $e$  and  $f$ , noting that  $(e, f) = 1$ . If

$$1 + bx^f = -ay^ez^f, \quad (7)$$

say, then it follows that

$$(z^{-1})^f + b(xz^{-1})^f = -ay^e,$$

which has the form (5). Conversely if (5) holds, then

$$1 + b(vw^{-1})^f = -a(w^{-1})^fu^e,$$

which has the form (7). Hence there is a one-to-one correspondence between the possible values  $u, v$  and  $w$  in (5), and the values  $x, y$  and  $z$  in (7). Since  $ef = p^n - 1$ , then there are  $e$  solutions of (7), except when there exists an  $x$  such that  $1 + bx^f = 0$ . In the latter case  $-bx^f = 1$ , and raising each member of this equation to the  $e$ th power, we have, since  $x^{ef} = x^{p^n-1} = 1$ , the condition  $(-b)^e = 1$ .

Hence we have

**THEOREM I.** *If  $ef = p^n - 1$ ,  $(e, f) = 1$ ,  $p$  is an odd prime then the number of solutions  $u^e, v^f, w^f$ , of the equation*

$$au^e + bv^f + w^f = 0; \quad abuvw \neq 0,$$

*with  $a, b, u, v$  and  $w$  quantities in the finite field of order  $p^n$ ,  $a$  and  $b$  given, is  $e$ , unless  $(-b)^e = 1$  in the field and then the number is  $e - 1$ .*

This is apparently a very special result, but if we apply the methods employed<sup>2</sup> (pp. 48-49) elsewhere we see that the theorem will give the exact number of solutions of the equation given, when  $e$  and  $f$  are replaced by any of their divisors, so our result has a variety of consequences. In our first paper we proved a Theorem I which gave a direct method for obtaining a residue of the number of solutions of (1) if the present paper modulo  $p$ . We were led to Theorem I of the present paper by pursuing this scheme originally. Now we shall write  $p^n = 1 + cl$  with  $l$  an odd prime and  $(c, l) = 1$ . To conform to notation used elsewhere, we shall, from now on, use  $g$  to denote a multiplicative generator of  $F(p^n)$  instead of  $\rho$ . If we have an element  $a$  of  $F(p^n)$  and  $g^k = a$  we write  $k = \text{ind } a$ . Consider the relation



$$g^{t+u} - 1 = g^{k+u}, \quad (8)$$

and denote the number of solutions in  $g^u, g^k$ , by  $(i, k)$ . We shall prove that

$$\text{ind } (g^u - 1) \equiv \sum_{h=1}^{l-1} h(i, h), \quad (9)$$

modulo  $l$ . From (8) there are  $(i, 1)$  solutions  $g^{u_1}$  such that

$$\text{ind } (g^{t+u_1} - 1) \equiv 1 \pmod{l};$$

and in general there are  $(i, j)$  solutions  $g^{u_j}$  such that

$$\text{ind } (g^{t+u_j} - 1) \equiv j \pmod{l}.$$

Now altogether the values of  $s_1, s_2, \dots, s_{l-1}$  are congruent to the  $l - 1$  numbers  $1, 2, 3, \dots, l - 1$ , modulo  $l$ . Hence

$$\sum_{j=0}^{l-1} \text{ind } (g^{t+u_j} - 1) \equiv \sum_{j=0}^{l-1} j(i, j), \quad (10)$$

modulo  $l$ . Now we have if  $A$  and  $B$  are elements,  $\neq 0$ , of  $F(p^n)$

$$\text{ind } A + \text{ind } B \equiv \text{ind } (AB) \pmod{l},$$

and the left-hand member of (10) may then be written

$$\text{ind } \left( \prod_j g^{u_j} \pi(g^t - g^{-u_j}) \right),$$

noting that

$$g^{t+u_j} - 1 = g^{-u_j}(g^t - g^{-u_j}).$$

We have

$$\prod_j g^{u_j} = g^{cm} = 1,$$

where  $m = (l - 1)/2$ . Also

$$\prod_j (g^t - g^{-u_j}) = (g^u - 1),$$

since  $g^{-u_j}$  satisfies  $x^l = 1$  in the field for any  $j$ . This gives (9).

In another paper the writer showed that if  $(a(a + 1), p) = 1$  then for some  $k$  in the set  $0, 1, \dots, l - 1$ , we have

$$\sum_{s=0}^{\infty} \binom{cr}{k + sl} a^{k+s} \equiv 1 \pmod{p},$$

and for any  $m$  in the set  $0, 1, \dots, l - 1$  with  $m \neq k$  we have

$$\sum_{s=0}^{\infty} \binom{cr}{m + sl} a^s \equiv 0 \pmod{p}.$$

Here

$$\binom{v}{w} = 0,$$

if  $w > v$ . We shall now extend the ideas in the proof of this by considering the special equation

$$g^{sd} + g^{tw} = g^{rt}, \quad (12)$$

in  $F(p^n)$ , where  $d = cl/(p-1)$ , and obtain other congruences involving the number of solutions of (11) and binomial coefficients. We also arrive at a number of congruences involving binomial coefficients alone. Take  $p$  to be a primitive root of  $l$  so that  $p^{l-1} - 1 = lc$ . We represent the finite field  $F(p^{l-1})$  by means of the set of residue classes with respect to  $\mathfrak{p}$ , a prime ideal factor of  $(p)$  in the algebraic field  $k(\zeta)$  where  $\zeta = e^{2\pi i/l}$ . Since  $p$  is a primitive root of  $l$  then  $\mathfrak{p} = (p)$  and from  $(g^{sd})^{p-1} \equiv 1 \pmod{\mathfrak{p}}$  we may replace  $g^{sd}$  by a non-zero rational integer, undetermined. Also we may select the generator (primitive root)  $g$  to be such that  $g^c \equiv \zeta \pmod{\mathfrak{p}}$ . Hence, the problem of finding the number of solutions of (12) is equivalent to the problem of finding the rational integers  $v$ ,  $t$  and  $r$  such that

$$1 + v\zeta^t \equiv \rho^r \pmod{\mathfrak{p}}, \quad (13)$$

where  $\rho$  is a primitive root of the congruence  $x^c \equiv 1 \pmod{\mathfrak{p}}$  in  $k(\zeta)$ .

We now consider the solutions of (13) with  $v$  fixed. There is at least one solution given by  $1 + v \equiv \rho_0 \pmod{\mathfrak{p}}$ , where  $\rho_0^c \equiv 1 \pmod{\mathfrak{p}}$ , since  $p-1 \not\equiv 0 \pmod{l}$  and hence any rational integer is congruent to an  $l$ th power modulo  $\mathfrak{p}$ . This may be the only solution of (13) for  $v$  fixed, but if there is another it must be of the type  $1 + v\zeta^a \equiv \rho_a$ ;  $\rho_a^c \equiv 1 \pmod{\mathfrak{p}}$ ;  $a \not\equiv 0 \pmod{l}$ . If in the last congruence involving  $v$  we use the substitution  $(\zeta^i/\zeta)$ ,  $i = 1, 2, \dots, l-1$  we obtain, since  $\mathfrak{p} = (p)$ , the relations

$$1 + v\zeta^i \equiv \rho_i, \quad (14)$$

$i = 0, 1, \dots, l-1$ ;  $\rho_i^c \equiv 1 \pmod{\mathfrak{p}}$ . If (14) exists then it is called an *A-set corresponding to  $v$* . Now consider the case where  $v = p-1$  in particular. Here  $1 + v \equiv 0$ , so that (14) does not exist for  $i = 0$ , but the other congruences in (14) may hold. If this is the case we say that there is a *B-set corresponding to  $p-1$* . If there is a  $v$ , say  $v_1$ , which does not satisfy (14) for  $i = 1$ , then there is just one relation involving  $v_1$ , namely

$$1 + v_1 = \rho'; \quad (\rho')^c \equiv 1 \pmod{\mathfrak{p}}$$

of the type (13). Then there is said to be a *C-set corresponding to  $v_1$* . Now take all the possible values of  $v$  in (13), namely  $1, 2, \dots, p-2$ , and also  $(p-1)$  provided  $t \not\equiv 0 \pmod{l}$ . Let  $n$  be the number of *A-sets* existing, then there are  $p-2-n$ , *C-sets*.

We shall derive a criterion for the existence of a  $B$ -set. In that case we have

$$1 + (p - 1)\zeta^t \equiv \rho; \rho^c \equiv 1$$

$$1 - \zeta^t \equiv \rho \pmod{p},$$

$$\zeta^{1/2}(\zeta^{-1/2} - \zeta^{1/2}) \equiv \rho \pmod{p},$$

and using the substitution  $(\zeta/\zeta^{-1})$  we have, if  $\rho_1^c \equiv 1$ ,

$$\zeta^{-1/2}(\zeta^{1/2} - \zeta^{-1/2}) \equiv \rho_1 \pmod{p},$$

whence

$$-\zeta \equiv \rho/\rho_1 \pmod{p},$$

and  $\zeta^c \equiv 1 \pmod{p}$ . And since  $p$  is prime to  $l$ , then  $c \equiv 0 \pmod{l}$ . But from the fact that this gives

$$\zeta^{-t/2} - \zeta^{t/2} \equiv \rho' \pmod{p}$$

we obtain, if  $(\rho')^c \equiv 1$ ,

$$1 - \zeta^t \equiv \zeta^{t/2}\rho' \pmod{p}$$

or

$$1 - (p - 1)\zeta^t \equiv \zeta^{t/2}\rho' \pmod{p}$$

Hence a necessary and sufficient condition for the existence of a  $B$ -set is that  $c \equiv 0 \pmod{l}$ .

We now assume that  $(c, l) = 1$  so that no  $B$ -set exists. In view of this there exist  $n$ ,  $A$ -sets and  $p - 2 - n$ ,  $C$ -sets so that the number of solutions  $N_0$  in  $v, \zeta, \rho$ , of (13) is

$$N_0 = p - 2 - n + nl. \quad (15)$$

We now proceed to find another expression for  $N_0$ . Using the method in another paper<sup>1</sup> we have where now  $i$  ranges over  $0, 1, \dots, p - 2$ ,

$$\sum_{i, t, s} (1 - (1 + h^t \zeta^i - \rho^s)^{p^{l-1}-1}) \equiv N, \quad (16)$$

modulo  $p$ , where  $h$  is a primitive root of  $p$  in the rational field,  $l$  ranges over the set  $0, 1, \dots, l - 1$ , and  $s$  over the set  $0, 1, \dots, c - 1$ . This reduces, using the methods of the same paper, modulo  $p$ , to

$$N_0 \equiv - \sum_{v=0}^{\infty} \sum_{k=1}^{l-1} \binom{kc}{ul(p-1)}. \quad (17)$$

In the same way we reduce the expression

$$N_0 = \sum_{i, t, s} (\zeta^i - \zeta^i(1 + h^t \zeta^i - \rho^s)^{p^{l-1}-1}),$$

where  $i, t$  and  $s$  range as in (16), which is congruent modulo  $p$  to the sum of the  $e$ th powers of all the possible values of  $\zeta$  in  $v, \zeta, \rho$ , of (13), modulo  $p$  to

$$N_e \equiv - \sum_{k=1}^{l-1} \sum_{u=0}^{\infty} \binom{kc}{ul(p-1)}, \quad (17a)$$

where  $s_e$  is selected so that  $s_e \equiv 0 \pmod{p-1}$  and also  $s_e \equiv -e \pmod{l}$  for any  $e$  such that  $0 \leq e < l$ . But if  $e \not\equiv 0 \pmod{l}$  the sum of the  $e$ -th powers of the solutions  $\zeta^t$  in (13) is easily seen to be  $p-2-n$ , so that

$$N_e \equiv -2 - n \pmod{p}, \quad (18)$$

$e = 1, 2, \dots, l-1$ . Eliminating  $n$  from the congruences (15) and (18) gives

$$N_0 + (l-1)N_e \equiv -2l \pmod{p} \quad (19)$$

$e \not\equiv 0 \pmod{l}$ .

We shall now find some other relations involving  $N_0$ . The expression  $1 + v\zeta^t$ , as  $v$  ranges over  $1, 2, \dots, p-1$  and  $t$  over  $0, 1, \dots, l-1$  independently can in general be expressed in the form  $\zeta^r \rho$ , where  $\rho^e \equiv 1$ , provided  $(c, l) = 1$ . Hence we have

$$(1 + v\zeta^t)^{fc} \equiv \zeta^d \pmod{p}. \quad (20)$$

Assume  $f \not\equiv 0 \pmod{l}$ . Then let  $v$  and  $t$  range as above indicated and add together the resulting congruences. Corresponding to each  $v$  for which an  $A$ -set exists the sum for these congruences on the right is  $l$ , so for all such  $v$ 's the sum on the right is  $nl$ . For a  $v$  such that we have a  $C$ -set the quantities on the right are  $1, \zeta, \zeta^2, \dots, \zeta^{l-1}$  in some order and the sum is zero. The other possibility is when  $v = p-1$  and then the sum on the right for this value of  $v$  is  $(-1)$ . When we add the congruences in (20) on the left for the various values of  $\zeta$  and  $t$  each term involving these is zero except those whose binomial coefficients have the form

$$\binom{fc}{ul(p-1)},$$

and we then find

$$(p-1)l \sum_{u=0}^{\infty} \binom{fc}{ul(p-1)} \equiv nl - 1,$$

modulo  $p$ . Since we have noted that (12) and (13) are equivalent, and using (15) we then obtain

**Theorem II.** *If  $p$  is a prime and a primitive root of the prime  $l$ ,  $p^{l-1} - 1 = lc$ ,  $(l, c) = 1$ ,  $g$  a primitive root of the finite field  $F(p^{l-1})$ , then the number of solutions in  $g^s, g^t, g^r$  of the equation*

$$g^{sd} + g^{td} = g^{rd}$$

in  $F(p^{l-1})$ , where  $d = cl/(p-1)$ , is  $p-2 + (l-1)n$ , where  $n$  is the least residue  $\geq 0$  of

$$-c - B_p$$

modulo  $p$ , and where

$$B_f = \sum_{u=0}^{\infty} \binom{fc}{ul(p-1)}.$$

Also, modulo  $p$ ,

$$B_1 \equiv B_a; \quad a = 1, 2, \dots, l-1.$$

<sup>1</sup> These PROCEEDINGS, 31, 170-172 (1945).

<sup>2</sup> *Ibid.*, 32, 47-52 (1946).

## A PARTIAL DIFFERENTIAL EQUATION OF FOURTH ORDER CONNECTED WITH RATIONAL FUNCTIONS OF A COMPLEX VARIABLE\*

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1. We shall consider the class of polynomials  $\phi(x, y)$  which are obtained as the numerator of the real part of a rational function of a complex variable  $z = x + iy$ . This new class contains the harmonic polynomials as a proper subset. In general, a polynomial  $\phi(x, y)$  of this new class does not satisfy the Laplace equation. We find that it does obey a certain partial differential equation of fourth order. Essentially we are dealing with the numerators of all rational solutions of the Laplace equation.

2. Harmonic polynomials are well known.<sup>1</sup> These are obtained as the real part of a polynomial or rational integral function in  $z$ . They constitute the class of polynomial solutions  $\phi(x, y)$  of the Laplace equation

$$\phi_{xx} + \phi_{yy} = 0. \quad (1)$$

3. Consider a rational function  $w = f(z)$  of the complex variable  $z$ . This is expressed as the quotient of two relatively prime polynomials in  $z$ . The degree  $r$  of  $f(z)$  is the maximum of the two degrees of the numerator and denominator.

Upon decomposing this rational function  $f(z)$  into its real and imaginary parts, we see that it can be written in the form

$$f(z) = \frac{\phi(x, y)}{D(x, y)} + i \frac{\psi(x, y)}{D(x, y)}, \quad (2)$$



where  $\phi(x, y)$ ,  $\psi(x, y)$ ,  $D(x, y)$  are real polynomials in the real variables  $(x, y)$  such that the numerator and denominator of each of the fractions appearing in the above equation have no common factors. Of course,  $D(x, y)$  can vanish only at the poles of  $f(z)$ .

The degree of  $\phi(x, y)$  (or  $\psi(x, y)$  or  $D(x, y)$ ) is  $2r - k$  where  $0 \leq k \leq r$ .

The new class of polynomials that we wish to study are all the polynomials  $\phi(x, y)$  (or  $\psi(x, y)$ ) which are obtained in this way.<sup>2</sup> Of course each of the component fractions in equation (2) satisfies the Laplace equation, but *the polynomials  $\phi(x, y)$ ,  $\psi(x, y)$ ,  $D(x, y)$ , do not obey the Laplace equation in general.* Obviously the harmonic polynomials are a proper subset of our new class.

4. FUNDAMENTAL THEOREM. *A polynomial  $\phi(x, y)$  is the numerator of the real (or imaginary) part of a rational function of a complex variable if and only if it obeys the partial differential equation of fourth order*

$$\left[ \frac{\partial^2}{\partial x^2} + \frac{\partial^2}{\partial y^2} \right] \log [\phi(\phi_{xx} + \phi_{yy}) - (\phi_x^2 + \phi_y^2)] = 0. \quad (3)$$

*In expanded form, this is*

$$\begin{aligned} & [\phi(\phi_{xx} + \phi_{yy}) - (\phi_x^2 + \phi_y^2)] [\phi_{xxxx} + 2\phi_{xxyy} + \phi_{yyyy}] \\ & - \phi[(\phi_{xxx} + \phi_{xyy})^2 + (\phi_{xxy} + \phi_{yyx})^2] + 2\phi_x[(\phi_{xx} - \phi_{yy})(\phi_{xxx} + \phi_{xyy}) + \\ & \quad 2\phi_{xy}(\phi_{xxy} + \phi_{yyx})] + 2\phi_y[2\phi_{xy}(\phi_{xxx} + \phi_{xyy}) \\ & - (\phi_{xx} - \phi_{yy})(\phi_{xxy} + \phi_{yyx})] - (\phi_{xx} + \phi_{yy})[(\phi_{xx} - \phi_{yy})^2 + 4\phi_{xy}^2] = 0. \end{aligned} \quad (4)$$

*Moreover, the bracketed expression of second order in (3) is not zero, and is always negative for real polynomials  $\phi(x, y)$ .*

The denominator  $D(x, y)$  satisfies this partial differential equation. As a matter of fact, it annuls the expression of second order appearing in (3). That is,  $D(x, y)$  is any polynomial solution of the partial differential equation of second order

$$\phi(\phi_{xx} + \phi_{yy}) - (\phi_x^2 + \phi_y^2) = 0. \quad (5)$$

5. *The polynomials  $\phi(x, y)$  and  $\psi(x, y)$  are the numerators of the real and imaginary parts of a rational function of a complex variable if and only if they obey the system of partial differential equations of second order*

$$\left. \begin{aligned} \phi(\phi_{xx} + \phi_{yy}) - (\phi_x^2 + \phi_y^2) &= \psi(\psi_{xx} + \psi_{yy}) - (\psi_x^2 + \psi_y^2), \\ \psi(\phi_{xx} + \phi_{yy}) + \phi(\psi_{xx} + \psi_{yy}) &= 2(\phi_x\psi_x + \phi_y\psi_y). \end{aligned} \right\} \quad (6)$$

In general, the two families of curves  $\phi(x, y) = \text{const.}$  and  $\psi(x, y) = \text{const.}$  are not orthogonal. However, the two algebraic curves  $\phi(x, y) = 0$  and  $\psi(x, y) = 0$  intersect orthogonally in  $[r^2 + (r - k)^2]$  points.

6. *The real and imaginary parts of the special class (M) of polygenic functions defined as the product of an analytic function of  $z = x + iy$  by*

another independent analytic function of  $\bar{z} = x - iy$ , constitute the totality of analytic solutions of our partial differential equation (3) of fourth order.

We observe that for this class ( $M$ ) of polygenic functions, the equations (6) play the rôle of the Cauchy-Riemann equations and the partial differential equation (3) or (4) of fourth order is the analogue of the Laplace equation.

\* Presented to the American Mathematical Society, February, 1947.

<sup>1</sup> The curves defined by setting a harmonic polynomial equal to zero were studied by Briot and Bouquet, Bôcher, and Kasner. See Briot and Bouquet, *Theorie des fonctions elliptiques*, Book IV, Chapter II, p. 226 (1875). Kasner, "On the Algebraic Potential Curves," *Bull. Amer. Math. Soc.*, **7**, 392-399 (1901). Also "Some Properties of Potential Surfaces," *Ibid.*, **8**, 243-248 (1902).

<sup>2</sup> The curves obtained by setting any polynomial  $\phi(x, y)$  of this class equal to zero, are being studied by the authors. See Kasner and De Cicco, "Rational Functions of a Complex Variable and Related Algebraic Potential Curves," these PROCEEDINGS, **32**, 280-282 (1946). See abstracts in *Bull. Amer. Math. Soc.*, 1946-1947.

## A REDUCTION THEOREM CONCERNING THE REPRESENTATION PROBLEM FOR FRÉCHET VARIETIES

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The object of this note is to prove a reduction theorem which will be employed in a forthcoming paper to solve the representation problem for a wide class of Fréchet surfaces. The reduction theorem itself is true in ultimate generality within the class of Fréchet varieties; consequently the discussion here is not restricted to Fréchet surfaces.

A first obligation is to define the terminology employed. If  $f_1(X^1) \subset Y \supset f_2(X^2)$  are mappings (= continuous transformations) from Peano spaces  $X^1$  and  $X^2$ , then  $f_1(X^1)$  is said to be *Fréchet equivalent* to  $f_2(X^2)$  if and only if, for every  $\epsilon > 0$  there is a homeomorphism  $h_\epsilon(X^1) = X^2$  such that  $\rho\{f_1(x^1), f_2h_\epsilon(x^1)\} < \epsilon$ , for  $x^1 \in X^1$ . (Notation:  $f_1 \sim f_2$ .) This equivalence relation partitions the totality of mappings from Peano spaces into equivalence classes  $[f]$ . Each equivalence class  $[f]$  is known as a *Fréchet variety*  $V$ , while any mapping in  $[f]$  is said to be a *representation* of the Fréchet variety  $V$ . It is clear that if  $f_1(X^1)$  and  $f_2(X^2)$  are representations of the same Fréchet variety  $V$ , then: (i) the range spaces  $X^1$  and  $X^2$  are topologically equivalent, (ii) the image spaces  $f_1(X^1)$  and  $f_2(X^2)$  are identical. Hence with each Fréchet variety  $V$  there is associated a pair of Peano spaces: the first,  $\tilde{V}$ , is topologically equivalent to the range space of any representation of  $V$ ; the second,  $|V|$ , is the common image

space of any representation of  $V$ . (The topological properties of  $\tilde{V}$  serve to catalogue Fréchet varieties; for example, if  $\tilde{V}$  is a 2-manifold,  $M$ , then  $V$  is said to be a Fréchet *surface* of the topological type of  $M$ .)

The *representation problem* for Fréchet varieties is the problem of determining the totality of representations of a Fréchet variety  $V$  upon being given any one representation,  $f$ , of  $V$ . In other words, the problem is to determine a *criterion* which will serve to obtain the totality of solutions,  $g$ , of the relation  $g \sim f$ . Such a criterion will be called an  $F$ -criterion. The importance of the problem is due to the fact that given one representation of a Fréchet variety there may be more favorable representations.

The definition of Fréchet equivalence itself, of course, serves as an  $F$ -criterion, but in applications it is often impossible to decide on the basis of the definition alone whether two given mappings are equivalent or not. Hence, the problem really asks for an effective solution, effective in the sense of its applicability.

For a short history of the problem one may consult a paper by Youngs.<sup>1</sup> Suffice it to say that the only solution to date is for the case in which  $V$  is of the topological type of a 2-sphere; that is,  $\tilde{V}$  is a 2-sphere. This note serves the purpose of recording a *reduction theorem* in the sense that the problem of finding an  $F$ -criterion for general mappings is reduced to the problem of finding an  $F$ -criterion for monotone mappings.

The statement of the reduction theorem requires a factor theorem due to Eilenberg and Whyburn. For a proof of the theorem, and the definitions of the terms involved, one may consult Whyburn,<sup>2</sup> pages 141–143.

**FACTOR THEOREM.** *If  $f(X) = Y$  is a mapping from a Peano space, then there is a monotone mapping  $m(X) = \mathfrak{X}$  and a light mapping  $l(\mathfrak{X}) = Y$  such that  $f(x) = lm(x)$ ,  $x \in X$ .*

A *monotone-light factorization* of  $f(X)$  is simply a factorization of the above type. (If  $f(X) = l_1m_1(X)$ ,  $m_1(X) = \mathfrak{X}^1$  and  $j(X) = l_2m_2(X)$ ,  $m_2(X^2) = \mathfrak{X}^2$  are two monotone-light factorizations, then there is a unique homeomorphism  $h(\mathfrak{X}^1) = \mathfrak{X}^2$  such that  $hm_1(x) = m_2(x)$ ,  $x \in X$ , and  $l_2h(\mathfrak{x}^1) = l_1(\mathfrak{x}^1)$ ,  $\mathfrak{x}^1 \in \mathfrak{X}^1$ .)

**REDUCTION THEOREM.** *Two mappings  $f_1(X^1)$  and  $f_2(X^2)$  from Peano spaces  $X^1$  and  $X^2$  are Fréchet equivalent if and only if there are monotone-light factorizations  $f_1(X^1) = lm_1(X^1)$  and  $f_2(X^2) = lm_2(X^2)$  such that  $m_1(X^1)$  and  $m_2(X^2)$  are Fréchet equivalent.*

*Proof.* Suppose first that there are monotone-light factorizations of the above type. Then  $m_1(X^1) = \mathfrak{X} = m_2(X^2)$ , and as  $l$  is continuous on the Peano space  $\mathfrak{X}$  it is uniformly continuous. Hence, for any  $\epsilon > 0$  there is a  $\delta$  such that  $\rho\{\mathfrak{x}_1, \mathfrak{x}_2\} < \delta$  implies  $\rho\{l(\mathfrak{x}_1), l(\mathfrak{x}_2)\} < \epsilon$ . Since  $m_1 \sim m_2$  there is a homeomorphism  $h_\delta(X^1) = X^2$  with the property that  $\rho\{m_1(x^1), m_2h_\delta(x^1)\} < \delta$ ,  $x^1 \in X^1$ . Therefore,  $\rho\{f_1(x^1), f_2h_\delta(x^1)\} = \rho\{lm_1(x^1), lm_2h_\delta(x^1)\} < \epsilon$ ,  $x^1 \in X^1$ , and so  $f_1 \sim f_2$ .

The converse follows as a direct consequence of a theorem of Radó<sup>3</sup> (page 425) or Youngs<sup>1</sup> (page 714). The development of Radó is to be preferred and will be used here. The basic statement is the following:

LEMMA OF RADÓ. *If  $lg_n(X)$ ,  $g_n(X) = \mathfrak{X}$ ,  $n = 1, 2, 3, \dots$  is a sequence of equicontinuous mappings from a Peano space  $X$  and the mapping  $l$  is light, then  $\{g_n\}$  is also a sequence of equicontinuous mappings.*

Since  $f_1 \sim f_2$ , for each  $n$  there is a homeomorphism  $h_n(X^1) = X^2$  such that  $\rho\{f_1(x^1), f_2h_n(x^1)\} < 1/n$ ,  $x^1 \in X^1$ . In other words,  $f_2h_n(x^1) \Rightarrow f_1(x^1)$ ,  $x^1 \in X^1$  ( $= f_2h_n(x^1)$  converges uniformly to  $f_1(x^1)$  on  $X^1$ ). Let  $f_2(X^2) = lm_2(X^2)$ ,  $m_2(X^2) = \mathfrak{X}$  be a monotone-light factorization. Now  $lm_2h_n(x^1) \Rightarrow f_1(x^1)$ ,  $x^1 \in X^1$ , and so  $\{lm_2h_n\}$  is a sequence of equicontinuous mappings. Consequently, by the lemma of Radó quoted above,  $\{m_2h_n\}$  is also a sequence of equicontinuous mappings. Since  $X$  is Peanian, the sequence  $\{m_2h_n\}$  contains a uniformly convergent subsequence, and it may be assumed that the notation  $\{m_2h_n\}$  refers to this subsequence, while  $m_1$  denotes its limit. It is important to notice that each mapping  $m_2h_n$  is monotone, consequently the statement  $m_2h_n(x^1) \Rightarrow m_1(x^1)$ ,  $x^1 \in X^1$  has several immediate implications.

(i)  $m_1 \sim m_2$ .

(ii)  $m_2h_n(X^1) = \mathfrak{X}$ , hence  $m_1(X^1) = \mathfrak{X}$  is monotone by a theorem of Whyburn<sup>2</sup> (page 174).

(iii)  $lm_2h_n(x^1) \Rightarrow lm_1(x^1)$ ,  $x^1 \in X^1$ . On the other hand,  $lm_2h_n(x^1) = f_2h_n(x^1) \Rightarrow f_1(x^1)$ ,  $x^1 \in X^1$ , and so  $f_1(x^1) = lm_1(x^1)$ ,  $x^1 \in X^1$ .

This shows that there are monotone-light factorizations  $f_1(X^1) = lm_1(X^1)$  and  $f_2(X^2) = lm_2(X^2)$  such that  $m_1 \sim m_2$ .

In conclusion it should be explicitly stated that the argument used in the second half of this theorem is the argument employed by Radó<sup>3</sup> (page 426). The only conclusion drawn here which is not mentioned by Radó is that  $m_1 \sim m_2$ . On the other hand, Radó proves a simple but fundamental statement (not recorded here) on the basis of which he is able to prove the most general theorems known concerning the cyclic additivity of the Lebesgue and de Geöcze areas of a Fréchet surface—a beautiful result. In view of these comments it is not unlikely that this reduction theorem, which will be of fundamental importance in the proposed solution of the representation problem, has apparently escaped mention because there was no intention to apply it. It is exhibited here as the key to a proposed body of research.

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<sup>1</sup> Youngs, J. W. T., "The Topological Theory of Fréchet Surfaces," *Annals Math.*, **45**, 753–785 (1944).

<sup>2</sup> Whyburn, G. T., "Analytic Topology," American Mathematical Society Colloquium Publications, Vol. 28 (1942).

<sup>3</sup> Radó, Tibor, "On Continuous Mappings of Peano Spaces," *Trans. Amer. Math. Soc.*, **58**, 420–454 (1945).

# ON SETS OF INTEGERS WHICH CONTAIN NO THREE TERMS IN ARITHMETICAL PROGRESSION

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Let  $S$  be a set of non-negative integers  $\leq N$  no three of which form an arithmetical progression (i.e.,  $A + A' \neq 2A''$  for any three distinct terms of  $S$ ). Let  $\nu(N)$  denote the maximum number of terms of such a "progression-free" set. Salem and Spencer<sup>1</sup> proved that for  $\epsilon > 0$  and sufficiently large  $N$

$$\nu(N) > N^{1 - \frac{\log 2 + \epsilon}{\log \log N}}.$$

I will show in this note that, by a modification of their method, the better estimate

$$\nu(N) > N^{1 - \frac{2\sqrt{2} \log 2 + \epsilon}{\sqrt{\log N}}}$$

can be obtained.

For any integers  $d \geq 2$ ,  $n \geq 2$ ,  $k \leq n(d-1)^2$  consider the set  $S_k(n, d)$  of all numbers of the form

$$A = a_1 + a_2(2d-1) + \dots + a_n(2d-1)^{n-1}$$

where the "digits"  $a_i$  are integers subject to the conditions

$$0 \leq a_i < d \tag{i}$$

$$(\text{norm } A)^2 = k \tag{ii}$$

where

$$\text{norm } A = \sqrt{a_1^2 + a_2^2 + \dots + a_n^2}.$$

This set is progression-free; for suppose  $A + A' = 2A''$  for  $A, A', A''$  in  $S_k(n, d)$  then

$$\text{norm } (A + A') = \text{norm } (2A'') = 2\sqrt{k}$$

and

$$\text{norm } A + \text{norm } A' = 2\sqrt{k}.$$

Thus, in the triangular inequality

$$\text{norm } (A + A') \leq \text{norm } A + \text{norm } A'$$

equality holds which is only possible if  $(a_1, a_2, \dots, a_n)$  and  $(a_1', a_2', \dots, a_n')$  are proportional and, as their norms are equal, identical, i.e., if  $A = A' = A''$ .



There are  $d^n$  different systems  $(a_1, a_2, \dots, a_n)$  satisfying (i) and  $n(d-1)^2 + 1$  possible values of  $k$ ; hence for some  $k = K$ ,  $S_k(n, d)$  must contain at least

$$\frac{d^n}{n(d-1)^2 + 1} > \frac{d^{n-2}}{n}$$

terms; as all these terms are  $< (2d-1)^n$  we have

$$\nu((2d-1)^n) > \frac{d^{n-2}}{n}.$$

Let  $N$  be given; choose  $n = \left\lceil \sqrt{\frac{2 \log N}{\log 2}} \right\rceil$ , and  $d$  such that

$$(2d-1)^n \leq N < (2d+1)^n.$$

Then,

$$\nu(N) \geq \nu((2d-1)^n) > \frac{d^{n-2}}{n} > \frac{(N^{1/n} - 1)^{n-2}}{n2^{n-2}} = \frac{N^{1-(2/n)}}{n2^{n-2}} (1 - N^{-1/n})^{n-2},$$

and, for sufficiently large  $N$ ,

$$\nu(N) > \frac{N^{1-(2/n)}}{n2^{n-1}} = N^{1 - \frac{2}{n} - \frac{\log n}{\log N} - \frac{(n-1)\log 2}{\log N}} > N^{1 - \frac{2\sqrt{2\log 2} + \epsilon}{\sqrt{\log N}}}$$

for any  $\epsilon > 0$ .

<sup>1</sup> Salem, R., and Spencer, D. C., "On Sets of Integers Which Contain No Three Terms in Arithmetical Progression," these PROCEEDINGS, 28, 561-563 (1942).

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## ALGEBRAIC TOPOLOGY AND INTEGRATION THEORY

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1. *Introduction.* We give here a preliminary account of a study of that side of integration theory which is useful particularly in differential geometry and fields centering around Stokes's theorem and the like. The subject is integration over  $r$ -dimensional domains (or, more generally, over  $r$ -chains) in an  $n$ -dimensional manifold or more general space (see below); the methods are direct, and are carried out largely without using coördinate systems. There are connections with real variable theory, but the problems are in general of a different nature. The methods are based on the fundamentals of algebraic topology; in fact, the work may be looked upon as the study of algebraic topology with real numbers as coefficients.

The integral  $\int_A X$  of a function  $X$  over a domain  $A$  corresponds to the "scalar" or "Kronecker" product  $X \cdot A$  of a cochain  $X$  and a chain  $A$ ; we shall write  $X \cdot A$  in place of  $\int_A X$ . The values of integrals are real numbers, but could equally well be elements of a Banach space. The work is largely a matter of proving inequalities; to make this possible, Lipschitz conditions are used throughout. In particular, cells  $\sigma$  used for domains of integration will be images of Euclidean cells  $\sigma_0$  under "Lipschitz mappings"  $f$ :

$$\text{dist}[f(p), f(q)] \leq N \text{dist}(p, q) \text{ for some } N.$$

The smallest possible such  $N$  is the *expansion*  $\xi(f, \sigma_0)$  of  $f$  in  $\sigma_0$ . (Though the inequalities depend on the metric in the space, the results are independent of Lipschitz changes of metric.) With this notion, a definition of the size  $|A|$  of a chain  $A$  is given; for usual domains, this is the  $r$ -dimensional volume of the domain. The integral corresponds to that of a bounded function:  $|\int_A X| \leq N|A|$  for some  $N$ . The extension to the unbounded case will not be considered here.

The most general space to which our results apply is a "Lipschitz space"  $R$ , defined as follows. It is a metric space (assumed compact for simplicity), for which there is an imbedding  $f$  into some Euclidean space  $E^n$ , both  $f$  and  $f^{-1}$  being Lipschitz. Further,  $f(R)$  is supposed "locally Lipschitz connected" in each dimension  $r$ . That is, for some  $\eta > 0$  and some  $N_r$ , any singular  $(r-1)$ -sphere  $S^{r-1}$  of diameter  $< \eta$  in  $f(R)$  bounds a singular  $r$ -cell  $Q'$  in  $f(R)$  such that

$$\text{diam } (Q') \leq N_r \text{ diam } (S^{r-1}).$$

There is then a retraction  $\rho$  of a neighborhood  $U$  of  $f(R)$  in  $E^n$  into  $f(R)$  such that  $\rho$  is Lipschitz.

2. *Lipschitz chains.* By  $\text{vol } (\sigma_0')$  we mean the usual  $r$ -volume of a Euclidean simplex  $\sigma_0'$  in metric Euclidean space. We define  $|\sigma'|$  for a Lipschitz singular simplex  $\sigma' = f(\sigma_0')$ , i.e., image of a Euclidean simplex  $\sigma_0'$  under a Lipschitz mapping  $f$ , as follows. Consider subdivisions  $\sigma_0' = \sum \sigma_{0i}'$  of  $\sigma_0'$  into Euclidean simplexes  $\sigma_{0i}'$ , and affine mappings  $g_i$  of Euclidean simplexes  $\tau_i'$  into  $\sigma_{0i}'$ ; set  $f_i(p) = f(g_i(p))$  in  $\tau_i'$ ; then

$$|\sigma'| = GLB \sum_i \mathcal{F}(f_i, \tau_i) \text{ vol } (\tau_i),$$

taking the greatest lower bound over all such expressions. For a Euclidean simplex or smooth simplex  $\sigma$ ,  $|\sigma| = \text{vol } (\sigma)$ . To extend the definition to Lipschitz singular chains  $A = \sum a_i \sigma_i' = \sum a_i f(\sigma_{0i}')$ , consider simultaneous subdivisions  $\sum_j \sigma_{0ij}'$  of the  $\sigma_{0i}'$ , and proceed as above, using  $|a_i|$ ; coefficients must be collected over the singular simplexes occurring.<sup>1</sup>

3. *Lipschitz cochains.* A Lipschitz  $r$ -cochain  $X'$  in  $R$  is a real-valued function  $X \cdot A'$  of Lipschitz singular chains  $A'$  in  $R$ , with the following properties: For some numbers  $N, N'$ ,

$$(a) \quad X \cdot (-\sigma) = -X \cdot \sigma,$$

$$(b) \quad |X \cdot \sigma| \leq N |\sigma|,$$

$$(c) \quad |X \cdot \partial \sigma| \leq N' |\sigma|.$$

Here,  $\partial \sigma$  denotes the boundary of an  $(r+1)$ -simplex  $\sigma$ . We let  $|X|$  and  $|\delta X|$  denote the least possible values of  $N$  and  $N'$ , respectively.  $X$ , defined for simplexes, becomes a linear function of Lipschitz singular  $r$ -chains by setting  $X \cdot \sum a_i \sigma_i' = \sum a_i X \cdot \sigma_i'$ . Now  $|X \cdot A| \leq |X| |A|$ .

The coboundary  $\delta X$  of  $X$  is defined by

$$\delta X' \cdot A'^{r+1} = X' \cdot \partial A'^{r+1}.$$

Written in the form  $\int_A \delta X = \int_{\partial A} X$ , this becomes Stokes's theorem.

If  $f$  is a Lipschitz mapping of  $R$  into  $R'$ , it carries cochains  $X' = X''$  of  $R'$  into cochains  $f^*X'$  of  $R$ , defined by  $f^*X' \cdot A = X' \cdot fA$ . We have  $\delta f^*X' = f^*\delta X'$ , and

$$|f^*X'| \leq \xi'(f) |X'|, |\delta f^*X'| \leq \xi'^{+1}(f) |\delta X'|.$$

A 0-cochain  $X$  is simply a point function:  $X(p) = X \cdot p$ . Its coboundary is given by  $\delta X \cdot (pq) = X \cdot \partial(pq) = X \cdot q - X \cdot p$ .

4. *Definition for polyhedral chains.* Let  $A = fA_0$  be a Lipschitz chain in  $R$ , expressed as the image of the chain  $A_0$  in the simplicial complex  $K_0$ . Let  $K_1$  be a simplicial subdivision of  $K_0$ . Let  $\mathfrak{S} A_0$  be a subdivision of  $A_0$ . Construct the cartesian product  $I \times K_0$  of the unit interval  $I = (0, 1)$  and  $K_0$ ; consider  $K_0$  as  $0 \times K_0$  and  $K_1$  as  $1 \times K_0$ . We may subdivide it simplicially so that

$$\partial \mathfrak{S}(I \times A_0) = \mathfrak{S}A_0 - A_0 - \mathfrak{S}(I \times \partial A_0).$$

Map  $I \times K_0$  into  $R$  by setting  $F(t, p) = f(p)$ . Then for each  $\tau$  of  $\mathfrak{S}(I \times A_0)$  not in  $0 \times K_0$  or  $1 \times K_0$ ,  $|F(\tau)| = 0$ . It follows from (b) and (c) that

$$X \cdot \mathfrak{S}A = X \cdot F\mathfrak{S}A_0 = X \cdot FA_0 = X \cdot A.$$

Thus  $X \cdot A$  is independent of subdivisions of  $A$ , and may be defined for "polyhedral singular chains"  $A$ .

Essentially the same method (with  $K_1 = K_0$ ) shows that  $X \cdot A$  is continuous under deformations of  $A$ .

For  $n$ -cochains  $X$  in  $E^n$  (or in an  $n$ -manifold) we may define  $X \cdot Q$  for open sets  $Q$ ; thus,  $X$  gives rise to a Lebesgue integral  $X \cdot Q = \int_Q D_x(p) dp$ , with the ordinary Lebesgue measure. Such a theorem is unknown for  $X^r$  in  $E^n$ ,  $1 < r < n$ .

5. *Special cochains; the de Rham Theorem.* For an oriented  $n$ -simplex  $\sigma^n$  in metric oriented  $E^n$ , let  $[\sigma^n]$  be  $\pm \text{vol}(\sigma^n)$  according as  $\sigma^n$  and  $E^n$  are oriented alike or unlike. Write  $I^n \cdot \sigma^n = [\sigma^n]$ ; then  $I^n$  is the unit  $n$ -cochain of metric oriented  $E^n$ . For any 0-cochain  $W^0$  and  $r$ -cochain  $X^r$ , we may define the  $r$ -cochain  $W^0 X^r$  by letting  $(W^0 X^r) \cdot \sigma^r = (W^0 \cdot p)(X^r \cdot \sigma^r)$  approximately, for  $p$  in  $\sigma^r$ , if  $\sigma^r$  is small; the definition is completed by a passage to the limit (using subdivisions of  $\sigma$ ). Now, with a notation from §4,  $X^n = D_x I^n$  in  $E^n$ .

A cochain  $X$  is a *cocycle* if  $\delta X = 0$ ;  $X$  and  $Y$  are *cohomologous* if  $Y - X = \delta Z$  for some  $Z$ . The classes of cohomologous cocycles are the elements of the  $r$ th *Lipschitz cohomology group* of the space.

In a simplicial complex  $K$ , to each Lipschitz cochain  $X$  corresponds an algebraic cochain  $\phi X$ , defined by  $\phi X \cdot \sigma_i^r = X \cdot \sigma_i^r$  for each  $r$ -simplex  $\sigma_i^r$  of  $K$ . This defines an isomorphism between the Lipschitz and the algebraic cohomology groups. This is the counterpart of the de Rham Theorem<sup>2</sup> (originally expressed in terms of homology theory). See also §§7 and 8.

In the proof, we define for each algebraic cochain  $X$  a Lipschitz cochain  $\psi X$  as follows. It is sufficient to define  $\psi X \cdot \tau$  for simplexes  $\tau$  lying in simplexes of  $K$  (compare §6). If we define  $\psi \sigma^r$  for each  $\sigma^r$ ,  $\psi X$  for all

$X$  will be defined. Take  $\sigma' = p_0 \dots p_r$ , and any  $\tau' = q_0 \dots q_r$  in a simplex of  $K$ . If  $\tau$  is not in the star of  $\sigma$ , set  $\psi\sigma \cdot \tau = 0$ . Otherwise, say  $\tau$  is in  $\sigma^* = p_0 \dots p_r p_{r+1} \dots p_s$ . Set

$$\psi\sigma \cdot \tau = [q_0 \dots q_r p_{r+1} \dots p_s] / [\sigma].$$

This is independent of the (Euclidean) metric employed. We may prove:  $\phi\psi X = X$ ,  $\delta\psi X = \psi\delta X$ .

6. *Lipschitz skeleton cochains.* A Lipschitz  $r$ -cochain  $X$  must have a value for all Lipschitz  $r$ -chains  $A$ ; these chains form a very large class of objects. We shall show that Lipschitz cochains may be derived from "Lipschitz skeleton cochains," defined for a much more restricted class of objects.

A *skeleton  $r$ -simplex*  $\sigma'$  in a metric space  $R$  is a set of  $r + 1$  points  $p_0, \dots, p_r$  in  $R$ . It is oriented like an abstract simplex. A *defining set of sides* of  $\sigma'$  is a set  $p_{\lambda_i} p_{\mu_i}$  such that if the  $p_i$  are put in  $E^r$ , the vectors  $v_i = p_{\mu_i} - p_{\lambda_i}$  ( $i = 1, \dots, r$ ) are independent. The *potentiality* of  $\sigma$  is

$$\text{Pot}(\sigma) = \frac{1}{r!} \min \text{dist}(p_{\lambda_1}, p_{\mu_1}) \dots \text{dist}(p_{\lambda_r}, p_{\mu_r}),$$

taking the minimum over defining sets of sides of  $\sigma$ .

A *Lipschitz skeleton cochain*  $x$  is a function  $x \circ \sigma$  such that  $(a') x \circ (-\sigma) = -x \circ \sigma$ , and

$$(b') |x \circ \sigma| \leq N \text{Pot}(\sigma), \quad (c') |x \circ \partial\sigma| \leq N' \text{Pot}(\sigma),$$

for some  $N, N'$ , as before. Again  $x \circ \sum a_i \sigma_i' = \sum a_i x \circ \sigma_i'$ .

We shall use a *standard* sequence of subdivisions<sup>2</sup>  $K_1, K_2, \dots$ , of a complex  $K_0$ : only a finite number of shapes of simplexes may occur in all the  $K_i$ . If  $A = fA_0$ ,  $A_0$  in  $K_0$ , and  $\mathfrak{S}_n A_0$  is the subdivided chain in  $K_n$ , etc., as in §4, then

$$\bar{x} \cdot A = \lim_{n \rightarrow \infty} x \circ f\mathfrak{S}_n A_0$$

exists, and  $\bar{x}$  is a Lipschitz cochain  $X$ , depending on  $x$  only. Conversely, any  $X$  gives rise to an  $x$  for which  $\bar{x} = X$ .

Skeleton cochains  $x, y$  are *equivalent* if  $\bar{x} = \bar{y}$ . This holds if for each  $\epsilon > 0$  there is a  $\delta > 0$  such that

$$|y \circ \sigma - x \circ \sigma| \leq \epsilon \text{Pot}(\sigma) \text{ if } \text{diam}(\sigma) < \delta.$$

7. *Tensor cochains.* For simplicity, we shall remain in  $E^n$ . A *Lipschitz tensor cochain* is a function  $T(p; v_1, \dots, v_r)$  of the point  $p$  and vectors  $v_1, \dots, v_r$ , which  $(a'')$  is linear and alternating in the vectors, and such that

$$(b'') |T(p; v_1, \dots, v_r)| \leq N |v_1| \dots |v_r|,$$

$$(c'') |T(p'; v_1, \dots) - T(p; v_1, \dots)| \leq N' |p' - p| |v_1| \dots |v_r|.$$



We may define  $T(p; V)$  where  $V$  is a contravariant  $r$ -vector, i.e., alternating product,  $v_1 \dots v_r$  (or a sum of such). For fixed  $p$ ,  $T$  is a covariant  $r$ -vector.

For a point function  $u(p)$  and a vector  $v$ , set

$$\nabla_v u(p) = \lim_{t \rightarrow 0} [u(p + tv) - u(p)]/t.$$

The covariant derivative of  $T$  is, if  $T$  is differentiable,

$$\delta T(p; v_1 \dots v_{r+1}) = \sum (-1)^{i-1} \nabla_{v_i} T(p; v_1 \dots \hat{v}_i \dots v_{r+1});$$

$\hat{v}_i$  denotes that this term is to be omitted.

To any oriented Euclidean simplex  $\sigma^r = p_0 \dots p_r$  in  $E^n$  corresponds a contravariant  $r$ -vector  $\{\sigma^r\}$ , defined by

$$\{\sigma^r\} = \frac{1}{r!} (p_1 - p_0) \dots (p_r - p_0);$$

if  $v_1, \dots, v_r$  is any defining set of sides of  $\sigma^r$ ,  $\{\sigma^r\} = \pm v_1 \dots v_r$ . The center of  $\sigma^r$  is  $p_\sigma = \Sigma p_i / (r + 1)$ .

To each  $T$  corresponds a Lipschitz skeleton cochain  $x = \Theta T$  defined by

$$\Theta T \cdot \sigma = T(p_\sigma; \{\sigma\}).$$

In the proof that (c') holds and for other matters, the following combinatorial theorem (corresponding to Stokes's theorem) is needed. Let  $L_0, \dots, L_r$  be covariant  $r$ -vectors. Let  $\sigma^r = p_0 \dots p_r$  be a simplex, with a defining set of sides  $v_i = p_{\mu_i} - p_{\lambda_i}$  ( $i = 1, \dots, r$ ). Let  $\sigma_i$  be the face of  $\sigma$  opposite  $p_i$ . Then

$$\sum_{i=0}^r (-1)^i L_i \{\sigma_i\} = \frac{1}{(r-1)!} \sum_{j=1}^r (-1)^j [L_{\mu_j}(\dots \hat{v}_j \dots) - L_{\lambda_j}(\dots \hat{v}_j \dots)].$$

Though  $\delta \Theta T \neq \Theta \delta T$  in general, they are equivalent (if  $T$  is differentiable); hence  $\delta \Theta T = \delta \Theta T = \Theta \delta T$ , as Lipschitz cochains. Using  $\Theta T$  defines an integration process without using coördinate systems.<sup>4</sup> With a coördinate system, the theory becomes ordinary integration; the theory of the Jacobian, Stokes's theorem, etc., follows simply.

The "tensor cohomology groups" again are isomorphic to the algebraic cohomology groups. (The de Rham Theorem is for the case that the tensors are differentiable.)

8. *Products of cochains.* The product of tensor cochains is well known. The corresponding product among Lipschitz cochains satisfies the following conditions:

( $\alpha$ )  $X^r Y^s$  is an  $(r + s)$ -cochain, linear in both  $X^r$  and  $Y^s$ .

( $\beta$ )  $X^0 Y^0$  is as defined in §5.

$$(\gamma) \quad |X \cdot Y| \leq N_r |X| |Y|.$$

$$(\delta) \quad \delta(X \cdot Y) = \delta X \cdot Y + (-1)^r X \cdot \delta X.$$

For general Lipschitz cochains, the products with these properties exist and are uniquely determined. (This holds in a Lipschitz space.) The usual properties hold:

$$(XY)Z = X(YZ); \quad Y \cdot X = (-1)^r X \cdot Y;$$

for Lipschitz mappings  $f$ ,  $f^*(XY) = (f^*X)(f^*Y)$ .

The product corresponds (under the operation  $\phi$  of §5) to the product<sup>5</sup>  $X \smile Y$  of algebraic cochains. Hence the cohomology rings in the algebraic, Lipschitz and tensor cases are isomorphic, completing the de Rham Theorem for these cases.

The fundamental tool for this section is the approximation to a cochain  $X$  by a set of coboundaries  $Z_i$ . Taking  $R$  in  $E^n$ , let  $\rho$  be the retraction of §1. Cut  $U$  into small pieces  $Q_i$ ; take  $p_i$  in  $Q_i$ , and set

$$Z_i \cdot \sigma^{r-1} = X \cdot \rho J(p_i, \sigma^{r-1})$$

for any  $\sigma^{r-1}$  in  $Q_i$ ;  $J$  denotes the join. Then  $|X - \delta Z_i|$  is small in  $Q_i$ .

9. *The continuity theorem.* A continuity theorem was mentioned in §4. We state a much more general (and difficult) theorem. Let  $A$  be a Lipschitz chain in  $R$ . For any  $\epsilon > 0$  and  $N$ , there is an  $\eta > 0$  with the following property. For any Lipschitz chain  $B$  satisfying  $|B| < N$ ,  $|\partial B| < N$ , and one further condition described below,

$$|X \cdot B - X \cdot A| \leq \max. (|X|, |\delta X|) \epsilon.$$

The further condition is: there exist chains (not necessarily Lipschitz)  $C$  within  $\eta$  of  $\partial A$  and  $D$  within  $\eta$  of  $A$  such that

$$B - A = \partial D + C.$$

Letting  $\eta \rightarrow 0$  is interesting: two chains  $A$  and  $B$  are "homology equivalent," i.e.,  $C$  and  $D$  exist for all  $\eta > 0$ , if and only if  $X \cdot A = X \cdot B$  for all  $X$ .

10. *Stokes's theorem.* With the help of methods described above (including the continuity theorem), a very general form of Stokes's theorem may be proved. We shall not give details here.

<sup>1</sup> See, for instance, Seifert-Threlfall, *Lehrbuch der Topologie*, Leipzig, 1934, Chapter IV.

<sup>2</sup> See G. de Rham, *Jour. de Math.* (10), 9, 115-200 (1931).

<sup>3</sup> See H. Freudenthal, *Annals Math.*, 43, 580-582 (1942).

<sup>4</sup> In an earlier version of this work, a large part of the theory was worked out by Paul Olum (1940, unpublished). See in this connection H. Whitney, *Duke Math. Jour.*, 4, 495-528 (1938), §§11, 12.

<sup>5</sup> See, for instance, H. Whitney, *Annals Math.*, 39, 397-432 (1938); see also H. Whitney, *Bull. Am. Math. Soc.*, 43, 785-805 (1937), p. 803.

# GEOMETRIC METHODS IN COHOMOLOGY THEORY<sup>1</sup>

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1. *Introduction.* In algebraic topology, the concepts of chains and cycles have considerable geometric content. Singular chains and cycles carry this content over to more general spaces such as polyhedra and absolute neighborhood retracts (*ANR's*). Cochains and cocycles, algebraically as simple as chains and cycles, did not appear until about 1935, in part because of the lack of a geometric interpretation. We give such an interpretation here;<sup>2</sup> it is, we believe, the natural one. Geometric cochains will be functions of singular chains in "general position."

The theory should apply to *ANR's*  $R$ ; at present, some of the basic theorems are proved only for polyhedra  $P$ . Full details will be given elsewhere. The coefficient group  $H$  will be supposed discrete. If  $H$  is continuous, for example, if it is the group of reals, the "nuclear system" (see below) may be discarded in whole or in part.<sup>3</sup>

2.  *$r$ -thin subsets of  $R$ .* A subset  $N$  of  $R$  is  *$r$ -thin* in  $R$  if any singular  $r$ -cell  $\sigma' = f(\sigma_0')$  in  $R$  may be pulled away from  $N$  by an arbitrarily small deformation  $f_1$ ;  $f_1(\sigma_0') \cap N = 0$ . For example, a  $k$ -plane in  $n$ -space  $E^n$  is  $(n-k-1)$ -thin in  $E^n$ . The set  $N$  and its complement may be very complicated.

Let  $f$  map the polyhedron  $P$  into  $P'$ ; let  $N$  be  $r$ -thin in  $P'$ . Then there is an arbitrarily small deformation  $f_1$  of  $f$  such that  $f_1^{-1}(N)$  is  $r$ -thin in  $P$ . (This has not been proved for *ANR's*).

Let  $N, N'$  be point sets in  $R$ . The singular chain  $A$  in  $R$  is in *general position* relative to  $(N, N')$  if  $A$  and  $\partial A$  do not intersect  $N'$  and  $N$ , respectively.

3. *Geometric cochains.* A geometric  $r$ - $H$ -cochain  $X = X'$  in  $R$  is a system of the following sort. It has a *nucleus*  $\bar{X}$  and a *nuclear boundary*  $\bar{X}' \subset \bar{X}$ ;  $\bar{X}$  and  $\bar{X}'$  are closed, and are  $(r-1)$ -thin and  $r$ -thin, respectively. For any singular  $r$ -chain  $A$ , with integer coefficients, in general position relative to  $(\bar{X}, \bar{X}')$ ,  $X \cdot A$  is defined, and is in  $H$ ; this is a homomorphism of the group of these chains into  $H$ . Further,

$$(a) \quad X' \cdot A' = 0 \text{ if } A' \text{ does not intersect } \bar{X},$$

$$(b) \quad X' \cdot \partial A'^{r+1} = 0 \text{ if } A'^{r+1} \text{ does not intersect } \bar{X}'.$$

The *coboundary*  $\delta X$  of  $X$ , with nuclear system  $(\bar{X}', 0)$ , is defined by  $\delta X' \cdot A'^{r+1} = X' \cdot \partial A'^{r+1}$ .

Clearly  $X$  is determined by its values for arbitrarily small singular cells near points of  $\bar{X} - \bar{X}'$ . Keeping all chains in general position,  $X \cdot A$  is easily proved independent of subdivisions and deformations of  $A$ .

If  $X \cdot \sigma = 0$  for all  $\sigma$  in some neighborhood  $U$  of  $p$  in  $\bar{X}$ , the points of  $\bar{X}$  in  $U$  may be dropped out. In this way, we obtain the *irreducible nucleus* of  $X$ . Note now that  $X \cdot A$  is always defined if  $\delta X = 0$ ,  $\partial A = 0$ .

For a simple example, let  $\bar{X}$  be a square in  $E^2$ ; let  $\bar{X}'$  be its boundary. Let  $u$  be a normal vector to  $\bar{X}$ . Then if  $A^1$  is a segment cutting through  $\bar{X}$ , let  $X^1 \cdot A^1$  be 1 or  $-1$  according as  $A^1$  is directed like  $u$  or unlike  $u$ . If  $A^2$  is a disc cutting  $\bar{X}'$ ,  $\delta X^1 \cdot A^2 = X^1 \cdot \partial A^2 = \pm 1$ .

Without further conditions, a cochain may be very complicated. For example, in  $E^n$  ( $n \geq 1$ ),  $X^n \cdot \sigma^n$  may have arbitrarily large values for arbitrarily small  $\sigma^n$  near a given  $p$  in  $X$ .

4. *Standard cochains in a complex.* In a complex  $K$  the "dual"  $\xi_r$  of a cell  $\sigma_r$  of  $K$  consists of simplexes of the first derived of  $K$ , of various dimensions in general; it forms a point set which is  $(r-1)$ -thin in  $K$ . It forms the nucleus of a cochain  $X_r' = \phi \sigma_r'$  such that  $X_r' \cdot \sigma_r' = \delta_H$ . Setting  $\phi \sum a_i \sigma_i' = \sum a_i X_i'$ , we have  $\delta \phi X = \phi \delta X$  for algebraic cochains  $X$  of  $K$ . These *standard cochains* operate on the algebraic chains of  $K$ , thought of as singular chains, just as the algebraic cochains do.

5. *Mappings and deformations.* Let  $f$  map  $P_1$  into  $P_2$ . Then given  $X_2$  in  $P_2$ , we may deform  $f$  into  $f_1$  (see §2) to make  $(f_1^{-1}(\bar{X}_2), f^{-1}(\bar{X}_2'))$  the nuclear system of a cochain  $X_1 = f_1^* X_2$  in  $P_1$ , defined by

$$f_1^* X_2 \cdot A_1 = X_2 \cdot f_1 A_1.$$

Then  $\delta f_1^* X_2 = f_1^* \delta X_2$ . If  $f_t(p)$  ( $p$  in  $P_1$ ,  $0 \leq t \leq 1$ ) is a deformation, such that  $f_0^* X_2$  and  $f_1^* X_2$  are defined, we may "deform" the deformation for  $0 < t < 1$ , and then define an  $(r-1)$ -cochain  $f_t^* X_2$ , such that

$$\delta f_t^* X_2 = f_1^* X_2 - f_0^* X_2 - f_t^* \delta X_2.$$

6. *A local property of cochains.* Given  $X'$  in  $R$  ( $r > 0$ ), and  $p$  in  $\bar{X} - \bar{X}'$ , let  $f_t$  be a deformation of the identity in  $R$  such that  $f_1(p)$  is not in  $\bar{X}$ . Say  $p$  is in  $U$ ,  $f_1(U) \cap \bar{X} = 0$ , and  $f_t(U) \cap \bar{X}' = 0$  for all  $t$ . Now  $f_0^* X = X$ ,  $f_1^* X = 0$  in  $U$ , and  $f_t^* \delta X = 0$  in  $U$ ; hence, by the relation above,  $X = \delta f_t^* X$  in  $U$ . Thus, *every geometric cochain of dimension  $> 0$  is locally a coboundary.*

7. *Geometric cohomology groups in a complex.* Let  $X'$  ( $r > 0$ ) be a geometric cochain in the complex  $K$  such that  $\delta X$  is standard (§4). With methods above, and other tools, we may show that there is a standard  $Y$  and a geometric  $Z$  such that  $X - Y = \delta Z$ . It follows that the geometric and algebraic cohomology groups of  $K$  are isomorphic.

8. *Properties of singular chains in terms of geometric cochains.* Conditions that a cochain  $X$  be 0, or a cocycle, or a coboundary, are expressible in a simple manner in terms of  $X \cdot A$  for various  $A$ ; we shall state some corresponding theorems for singular chains. Say  $A_1$  and  $A_2$  are *essentially equal*:  $A_1 = A_2$ , if, for arbitrary neighborhoods  $U$  of  $A_1 \cup A_2$  and  $U'$

of  $\partial A_1 \cup \partial A_2$ , there are singular chains  $B \subset U$  and  $C \subset U'$  such that  $A_1 - A_2 = \partial B + C$ . (This relation is not transitive, unless some assumption to eliminate chains with space-filling characteristics is made.) Now the conditions  $A = 0$ ,  $\partial A = 0$ ,  $A = \partial B$  for cycles  $A$ , are equivalent to  $X \cdot A = 0$ ,  $\delta X \cdot A = 0$ ,  $X \cdot A = 0$  if  $\delta X = 0$ , for all cochains  $X$  of the proper dimension in general position relative to  $A$ .

9. *Products of geometric cochains.* We first give an example. Let  $\bar{X}$  and  $\bar{Y}$  be squares in  $E^3$ , as in §3; let them intersect in a segment  $\bar{Z}$ . (Do not let  $\bar{X}'$  and  $\bar{Y}'$  intersect.) Take  $p$  in  $\bar{Z}$ ; let  $u$  and  $v$  be vectors at  $p$  normal to  $\bar{Z}$ , and lying in  $\bar{Y}$  and  $\bar{X}$ , respectively. If  $A^1$  is a segment cutting  $\bar{X}$  in the direction of  $u$ , say  $X \cdot A^1 = \alpha$ ; define  $\beta$  similarly for  $\bar{Y}$  and  $v$ . Then if  $A^2$  is a disc cutting  $\bar{Z}$  at  $p$  and containing  $u$  and  $v$ , and  $A^2$  is oriented by the ordered pair  $(u, v)$ , set  $Z^2 \cdot A^2 = \alpha\beta$ . We set

$$Z = X \smile Y; \bar{Z} = \bar{X} \cap \bar{Y}, \bar{Z}' = (\bar{X} \cap \bar{Y}') \cup (\bar{X}' \cap \bar{Y}).$$

We now discuss the general situation. Say  $X^r$  and  $Y^s$  are in *general position* if  $\bar{Z}$  and  $\bar{Z}'$  defined above are  $(r + s - 1)$ -thin and  $(r + s)$ -thin, respectively, and  $\bar{X}' \cap \bar{Y}'$  is  $(r + s + 1)$ -thin. (Given arbitrary  $X$  and  $Y$ , slight deformations  $f_i$  and  $g_i$  of the identity  $f_0 = g_0$  will bring  $f_1^*X$  and  $g_1^*Y$  into general position.) Given any  $p$  in  $\bar{Z} - \bar{Z}'$ , define  $Z$  in a small neighborhood  $U$  of  $p$  as follows. First, if  $r = 0$ ,

$$Z^1 \cdot \sigma^0 = (X^0 \cdot p)(Y^0 \cdot \sigma^0), \sigma^0 \text{ in } U.$$

For  $r > 0$ , say  $X = \delta X_1$ ,  $\delta Y = 0$ , in  $U$  (see §6); using induction, set

$$Z^{r+s} \cdot \sigma^{r+s} = (X_1^{r-1} \smile Y^s) \cdot \partial \sigma^{r+s}, \sigma^{r+s} \text{ in } U.$$

The proof of existence and uniqueness, together with the usual properties of the product, is carried out with the help of induction.

10. *The usual products in a simplicial complex.* If  $K$  is a simplicial complex with ordered vertices, there is a simple way of defining two systems of duals  $\xi_i^r, \eta_j^s$  (see §4) so that any corresponding  $\varphi_i X^r, \varphi_j Y^s$  are always in general position.<sup>4</sup> Now for any algebraic  $X^r$  and  $Y^s$ , and any simplex  $\sigma^{r+s}$  of  $K$ , which we may think of as a singular simplex,

$$(\varphi_i X^r \smile \varphi_j Y^s) \cdot \sigma^{r+s}$$

is defined, and is the same as the usual algebraic product  $(X \smile Y) \cdot \sigma^{r+s}$ .

<sup>1</sup> Presented to the Am. Math. Society, Feb. 21, 1941, under the title "Geometric methods in combinatorial topology."

<sup>2</sup> For earlier work along these lines, see S. Wylie, *Proc. London Math. Soc.*, 46, 174-198 (1940). An application will be found in H. Whitney, *Annals Math.* (2), 45, 220-246 (1944), §5.

<sup>3</sup> The theory then takes on a completely different form. See the preceding paper in these PROCEEDINGS.

<sup>4</sup> Compare H. Whitney, these PROCEEDINGS, 26, 143-148 (1940), §5.



COMPLEXES OF MANIFOLDS<sup>1</sup>

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1. *Introduction.* A smooth manifold  $M$  (smooth means continuously differentiable) is a well-defined concept. A bounded manifold may have, for boundary, anything from a smooth manifold to quite a general point set. For analytical purposes, the boundary should at least be made up of pieces of manifolds, joining together smoothly; that is, it should be formed of a "complex of manifolds," or "complifold" for short. Then  $M = \sigma^n$ , together with the boundary cells  $\sigma_i^r$ , forms a complifold  $K$ . If one tries to define  $K$  abstractly, one finds that all sorts of curious situations may occur locally. In this preliminary note, we wish to point out some of the properties that will bring  $K$  back to reasonableness. If  $K$  is imbeddable in a Euclidean space  $E^m$ , each cell being imbedded smoothly, this should be satisfactory.

2. *Complifolds.* Instead of giving a full definition, we shall point out some of the properties that are clearly necessary. The cells should attach to each other, in the point set sense, like the cells of an ordinary complex. The relation between coördinate systems in incident cells should be smooth, with Jacobian matrix of maximum rank. If a vector is tangent to faces  $\sigma_1, \sigma_2$  of a cell  $\sigma$  at  $p$ , it should be tangent to a common face of  $\sigma_1$  and  $\sigma_2$ .

Among the important subjects we cannot discuss here, we mention the following. The property of  $K$  being "locally flat" or "locally simplicial"; the property of  $K_1$  and  $K_2$  being in "general position" in  $K$ ; the fact that, in this case,  $K_1 \cap K_2$  is a complifold.

The local properties of  $K$  can be described largely in terms of tangent vectors, to which we now turn.

3. *The tangent space of  $K$  at a point.* For each (closed) cell  $\sigma$ , and point  $p$  in  $\sigma$ , there is a *tangent cone*  $\Gamma(\sigma, p)$  of vectors  $(\sigma, v)$  which are tangent to  $\sigma$  at  $p$ ; these generate a vector space  $V(\sigma, p)$ . Given  $p$  in  $K$ , let  $\sigma_1, \dots, \sigma_r$  be the cells containing  $p$ . Define (abstractly) the direct sum

$$V^*(p) = V(\sigma_1, p) \oplus \dots \oplus V(\sigma_r, p).$$

The vectors

$$w_{ij}(v) = (\sigma_i, v) - (\sigma_j, v), \quad v \text{ in } V(\sigma_i, p) \cap V(\sigma_j, p)$$

generate a subspace  $V^{**}(p)$  of  $V^*(p)$ . The *tangent space*

$$V(K, p) = V^*(p) \ominus V^{**}(p)$$

of  $K$  at  $p$  is formed from  $V^*(p)$  by setting all  $w_{ij}(v) = 0$ .

We say  $K$  is *consistent at  $p$*  if each  $\Gamma(\sigma, p)$  lies in  $V(K, p)$  in a one-one manner. This is obviously a necessary condition that  $K$  be smoothly imbeddable in  $E^m$ .

It may happen that a relation  $a_1v_1 + \dots + a_kv_k = 0$  is not true at  $p$ , yet arbitrarily near  $p$  a relation arbitrarily near this one holds. This might preclude smooth imbedding.

A necessary and sufficient condition for imbeddability of a general complifold seems highly difficult to obtain. Instead, we shall give a slight restriction on  $K$ .

4. *Cellwise homogeneity.* Let  $K$  be consistent at each  $p$ . If for each  $\sigma$ , and cells  $\sigma_1, \dots, \sigma_n$ , with  $\sigma$  as face, the part of  $V(K, p)$  over these cells is of constant dimension as  $p$  moves over  $\sigma$ , we say  $K$  is *cellwise homogeneous*.

**THEOREM:** *Any cellwise homogeneous complifold of dimension  $n$  may be smoothly imbedded in  $E^{2n+1}$ , and smoothly immersed in  $E^{2n}$ .*

<sup>1</sup> Presented to the American Mathematical Society Sept. 8, 1942.

## ON TOTALLY POSITIVE FUNCTIONS, LAPLACE INTEGRALS AND ENTIRE FUNCTIONS OF THE LAGUERRE-POLYA-SCHUR TYPE

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The purpose of this note is to furnish a solution of the problem of representing by Laplace integrals the reciprocals of entire functions with real zeros only, which are of genus zero or one up to certain exponential factors. The solution appears in terms of a certain class of functions which we propose to call *totally positive functions*.

1. *Totally Positive Functions.*—A real-valued function  $\Lambda(x)$ , defined for all real  $x$ , is said to be *totally positive* (abbreviated: t. p.) if it satisfies the following three conditions:

( $\alpha$ )  $\Lambda(x)$  is measurable.

( $\beta$ ) If  $x_1 < x_2 < \dots < x_n$  and  $t_1 < t_2 < \dots < t_n$ , then the determinant of order  $n$ , whose element in the  $i$ th row and  $j$ th column is  $\Lambda(x_i - t_j)$ , should be non-negative, i.e.,

$$\det \|\Lambda(x_i - t_j)\| \geq 0. \quad (1)$$

This inequality should be verified for  $n = 1, 2, 3, \dots$

( $\gamma$ )  $\Lambda(x)$  should be positive for at least two distinct values of  $x$ .

For  $n = 1$ , the inequality (1) is equivalent to  $\Lambda(x) \geq 0$  for all real  $x$ , justifying the name of total positivity for our set of conditions.

A trivial example of t. p. functions is furnished by

$$\Lambda(x) = e^{ax+b}. \quad (2)$$

Indeed, all determinants (1) vanish if  $n \geq 2$ . Another t. p. function is

$$\Lambda(x) = \begin{cases} e^{-x} & \text{if } x \geq 0 \\ 0 & \text{if } x < 0. \end{cases} \quad (3)$$

Indeed, we readily find in this case that<sup>1</sup>

$$\det \|\Lambda(x_i - t_j)\| = \exp\{-\sum x_i + \sum t_j\} \cdot \epsilon,$$

where  $\epsilon = 1$  or  $\epsilon = 0$ , depending on whether or not all inequalities  $t_1 \leq x_1 < t_2 \leq x_2 < t_3 \leq x_3 < \dots < t_{n-1} \leq x_{n-1} < t_n \leq x_n$  hold simultaneously. Incidentally, with  $\Lambda(x)$  defined by (3), the functions  $\Lambda(ax + b)$  ( $a \neq 0$ ) are the only discontinuous t. p. functions. A further noteworthy example is

$$\Lambda(x) = e^{-x^2}. \quad (4)$$

The total positivity of this function is likewise clear because

$$\det \|\exp\{-(x_i - t_j)^2\}\| = \exp\{-\sum x_i^2 - \sum t_j^2\} \cdot \det \|\exp\{2x_i t_j\}\|,$$

the last determinant being known to be positive.<sup>2</sup>

It is easy to see that of the conditions  $(\alpha)$ ,  $(\beta)$ ,  $(\gamma)$ , neither one is implied by the other two. Thus, if

$$\Lambda(x) = \exp\{-\psi(x)\}, \quad (5)$$

where  $\psi(x)$  is a discontinuous, hence non-measurable, solution of  $\psi(x + y) = \psi(x) + \psi(y)$ , then the conditions  $(\beta)$  and  $(\gamma)$  are fulfilled without  $(\alpha)$  holding.

The conditions  $(\alpha)$  and  $(\gamma)$ , together with the inequality (1), for  $n = 1$  and  $n = 2$  only, are found to be equivalent with the requirement that the function  $\psi(x)$ , of (5), is a convex function. This remark readily leads to the following proposition: *For any t. p. function  $\Lambda(x)$  we have*

$$0 < \int_{-\infty}^{\infty} \Lambda(x) dx \leq +\infty.$$

*A t. p. function  $\Lambda(x)$  is either monotone (in the wide sense), in which case*

$$\int_{-\infty}^{\infty} \Lambda(x) dx = +\infty, \quad (6)$$

*or else it is not a monotone function, in which case we have*

$$0 < \int_{-\infty}^{\infty} \Lambda(x) dx < +\infty. \quad (7)$$

A non-monotone t. p. function  $\Lambda(x)$  may therefore also be thought of as a frequency function. We wish to call them *Polya frequency functions*. Among our examples of t. p. functions we find that (2) is monotone, while

(3) and (4) are Polya frequency functions. A study of the class of t. p. functions reduces essentially to a study of its subclass of Polya frequency functions in view of the following proposition: *If  $\Lambda(x)$  is t. p. then either  $\Lambda(x) = \exp\{ax + b\}$ , or else there is a number  $\omega$  such that  $\Lambda_0(x) = e^{\omega x} \cdot \Lambda(x)$  is a Polya frequency function.*

2. *Entire functions of the Laguerre-Polya-Schur type.*—Following Polya and Schur<sup>3</sup> we shall say that an entire rational or transcendental function  $\Phi(z)$  is an *entire function of type I*, if its canonical representation is of the form

$$\Phi(z) = Cz^n e^{\gamma z} \prod_{\nu=1}^{\infty} (1 + \delta_{\nu} z), \quad (C \geq 0, n \geq 0, \gamma \geq 0, \delta_{\nu} \geq 0). \quad (8)$$

Likewise we shall say that  $\Psi(z)$  is an *entire function of type II* if its canonical representation is of the form

$$\Psi(z) = Cz^n e^{-\gamma z} \prod_{\nu=1}^{\infty} (1 + \delta_{\nu} z) e^{-\delta_{\nu} z}, \quad (C \leq 0, n \geq 0, \gamma \geq 0, \gamma, \delta_{\nu} \text{ real}). \quad (9)$$

It was shown by Laguerre and Polya<sup>4</sup> that the entire functions of type I *and no others* are the uniform limits ( $\neq 0$ ) of real polynomials having all their roots on the half-line  $x \leq 0$  ( $z = x + yi$ ). Likewise that the entire functions of type II *and no others* are the uniform limits ( $\neq 0$ ) of real polynomials with only real roots. The coefficients of the power series expansions of the functions  $\Phi(z)$  and  $\Psi(z)$  about the origin enjoy remarkable algebraic properties which were discovered by Polya and Schur (*loc. cit.*) on the foundation of Laguerre's pioneering work. Finally, Polya<sup>5</sup> investigated the expansion coefficients of the reciprocals  $1/\Phi(z)$  and  $1/\Psi(z)$  about the origin. His results suggested that these reciprocals should allow of representations by the ordinary Laplace-Stieltjes integral and by the bilateral Laplace-Stieltjes integral, respectively, both with monotone determining functions. This problem was later investigated by H. Hamburger.<sup>6</sup> However, Hamburger's results do not exhibit the exact nature of the Laplace integral representations of these particular classes of functions.

3. *Laplace integrals.*—The relationship between the reciprocals of functions of the Laguerre-Polya-Schur type and the Laplace integral is described by the following theorem.

**THEOREM 1.** *Let  $\Lambda(x)$  be a totally positive function which is not of the form  $\exp\{ax + b\}$ . Then the bilateral Laplace integral*

$$\int_{-\infty}^{\infty} e^{zx} \Lambda(x) dx \quad (10)$$

*converges in a strip  $\alpha < \operatorname{Re} z < \beta$  ( $-\infty \leq \alpha < \beta \leq +\infty$ ) and represents there the reciprocal*

$$\frac{1}{\Psi(z)} \quad (11)$$

of an entire function  $\Psi(z)$  of type II which is not of the form  $C \cdot e^{bz}$ . The endpoints  $\alpha, \beta$  of the strip of convergence of the integral (10) are zeros of  $\Psi(z)$ , provided they are finite.

Conversely, let (11) be the reciprocal of a function (9) of type II, not of the form  $C \cdot e^{bz}$ , and let  $\alpha < Rz < \beta$  ( $-\infty \leq \alpha < \beta \leq +\infty$ ) be a strip in which (11) is regular. Then  $1/\Psi(z)$  (if  $\Psi(z) > 0$  in  $\alpha < z < \beta$ ), or else  $-1/\Psi(z)$  (if  $\Psi(z) < 0$  in  $\alpha < z < \beta$ ), may be there represented by a Laplace integral (10), where  $\Lambda(x)$  is a totally positive function.

We, therefore, have a 1-1 correspondence between t. p. functions and reciprocals of functions of type II in a given strip of regularity  $\alpha < Rz < \beta$  ( $-\infty \leq \alpha < \beta \leq \infty$ ) by the relation

$$\int_{-\infty}^{\infty} e^{xz} \Lambda(x) dx = \frac{1}{\Psi(z)} \cdot \epsilon, \quad (\alpha < Rz < \beta), \quad (12)$$

where  $\epsilon = \operatorname{sgn} \Psi(z)$  in  $\alpha < z < \beta$ .

Further properties of the correspondence (12) are as follows.

**THEOREM 2.** *The totally positive function  $\Lambda(x)$  is a Polya frequency function if and only if*

$$\alpha < 0 < \beta \quad (13)$$

Otherwise  $\Lambda(x)$  is always monotone, namely, non-increasing if  $0 \leq \alpha$  and non-decreasing if  $\beta \leq 0$ .

**THEOREM 3.** *The relation (12) may always be inverted by Mellin's integral to*

$$\Lambda(x) = \frac{1}{2\pi i} \int_{\sigma - \infty i}^{\sigma + \infty i} \frac{\epsilon}{\Psi(z)} e^{-xz} dz, \quad (\alpha < \sigma < \beta, -\infty < x < \infty). \quad (14)$$

Unless  $\Psi(z)$  is free of zeros, there are several t. p. functions  $\Lambda(x)$  associated with the same  $\Psi(z)$ , corresponding to the various strips of regularity of its reciprocal. The way in which these various  $\Lambda(x)$  are connected with each other is as follows.

**THEOREM 4.** *Let  $(\alpha, \beta)$  and  $(\beta, \gamma)$  be contiguous intervals of regularity of  $1/\Psi(z)$  ( $-\infty \leq \alpha < \beta < \gamma \leq \infty$ ) and let  $\Lambda(x)$  and  $\Lambda_1(x)$  be the corresponding totally positive functions, i.e.,*

$$\frac{\epsilon}{\Psi(z)} = \int_{-\infty}^{\infty} e^{xz} \Lambda(x) dx \quad (\alpha < Rz < \beta), \quad \frac{\epsilon_1}{\Psi(z)} = \int_{-\infty}^{\infty} e^{xz} \Lambda_1(x) dx \quad (\beta < Rz < \gamma),$$

where  $\epsilon = \pm 1$ ,  $\epsilon_1 = \pm 1$ , such that  $\epsilon \Psi(z) > 0$  if  $\alpha < z < \beta$ , and  $\epsilon_1 \Psi(z) > 0$  if  $\beta < z < \gamma$ . Then



$$\epsilon_1 \Lambda_1(x) - \epsilon \Lambda(x) = \text{The residue of } e^{-xz}/\Psi(z) \text{ at } z = \beta, \quad (15)$$

for all real values of  $x$ .

Functions  $\Phi(z)$  of type I being at the same time also functions of type II, the theorems just stated apply to such functions as well. In this particular case, however, we may add to Theorem 1 the following additional information.

THEOREM 5. Let  $\Lambda(x)$  be a Polya frequency function such that

$$\Lambda(x) = 0 \text{ if } x > 0. \quad (16)$$

Then

$$\int_{-\infty}^0 e^{xz} \Lambda(x) dx = \frac{1}{\Phi(z)}, \quad (Rz > \alpha, \text{ for some } \alpha < 0) \quad (17)$$

where  $\Phi(z)$  is an entire function of type I, with  $\Phi(0) > 0$ , and not of the form  $C \cdot e^{\delta z}$ . Conversely, if  $\Phi(z)$  is of type I,  $\Phi(0) > 0$ , not of the form,  $C \cdot e^{\delta z}$ , then it may be represented by the ordinary Laplace integral (17), where  $\Lambda(x)$ , if defined as  $= 0$  for  $x > 0$ , is a Polya frequency function.

4. Examples.—A few classical integral representations will serve to illustrate the theory. The following formulae are due to Euler:

$$\int_{-\infty}^{\infty} e^{xz} e^{-e^x} dx = \Gamma(z) \quad (Rz > 0), \quad (18)$$

$$\int_{-\infty}^{\infty} e^{xz} \frac{1}{1 + e^x} dx = \frac{\pi}{\sin \pi z} \quad (0 < Rz < 1), \quad (19)$$

$$\int_{-\infty}^0 e^{xz} (1 - e^x)^{n-1} e^x dx = \frac{(n-1)!}{(z+1)(z+2)\dots(z+n)} \quad (Rz > -1). \quad (20)$$

The right-hand sides are reciprocals of functions of type II, hence the determining functions  $\Lambda(x)$  are all totally positive by Theorem 1' (converse part). The direct part of Theorem 1 may at times also be applied and that should be the direction of the more significant applications, if any. To illustrate this point, let us prove (the well-known fact) that  $1/\Gamma(z)$  is of type II. By (18) it is sufficient to show that  $\Lambda(x) = \exp \{-e^x\}$  is totally positive. But this is clear since

$$\det \|\Lambda(x_i - t_j)\| = \det \|\exp \{-e^{x_i} \cdot e^{-t_j}\}\| = \det \|\exp \{\alpha_i \beta_j\}\| > 0,$$

since both sequences of numbers

$$\alpha_i = e^{x_i}, \beta_i = -e^{-t_i} \quad (i = 1, 2, \dots, n)$$

are monotone increasing. A similar, equally simple argument applies to (19) showing that  $\sin(\pi z)$  is of type II.

In order to illustrate Theorem 4 we inquire into the integral representation

$$\int_{-\infty}^{\infty} e^{xz} \Lambda_n(x) dx = (-1)^{n+1} \Gamma(z), \quad (-n-1 < Rz < -n), \quad (n > 0), \quad (21)$$

which is assured by Theorem 1. Starting from the representation (18), the various transitional residues appearing in (15) are readily determined with the result that

$$\Lambda_n(x) = (-1)^{n+1} \left\{ e^{-x^2} - \left( 1 - \frac{1}{1!} e^x + \frac{1}{2!} e^{2x} - \dots \pm \frac{1}{n!} e^{nx} \right) \right\},$$

$$(-\infty < x < \infty). \quad (22)$$

This function is totally positive by Theorem 1.

Formula (20) illustrates Theorem 5. Another example to Theorem 5 is furnished by the formula

$$\int_0^{\infty} e^{xz} dL(x) = \prod_{n=1}^{\infty} \frac{1}{1 - \frac{z}{n^2}}, \quad (Rz < 1), \quad (23)$$

where the distribution function  $L(x)$  is defined by

$$L(x) = \vartheta_0 \left( 0 \mid \frac{ix}{\pi} \right) = \sum_{n=-\infty}^{\infty} (-1)^n e^{-xn^2}, \quad (x > 0, L(0) = 0).$$

Its derivative

$$\Lambda(x) = \begin{cases} \sum_{n=-\infty}^{\infty} (-1)^{n+1} n^2 e^{-xn^2} & \text{if } x > 0, \\ 0 & \text{if } x \leq 0, \end{cases} \quad (24)$$

is, by Theorem 5, a Polya frequency function.

5. *Concluding remarks.*—1. Various other properties of a t. p. function  $\Lambda(x)$  are reflected in corresponding properties of the associated function  $\Psi(z)$  of (12). Thus  $\Lambda(x)$  is of class  $C^{\infty}$  if, and only if, in (9) we have either  $\gamma > 0$ , or else  $\gamma = 0$  and  $\delta_\nu > 0$  for infinitely many  $\nu$ . Thus the  $\Lambda(x)$  of (24) is of class  $C^{\infty}$ , i.e., all derivatives of  $\Lambda(x)$  vanish at the origin. However, if  $\gamma = 0$  and  $\Psi(z)$  is a polynomial of degree  $m(> 0)$  multiplied by an exponential factor, then  $\Lambda(x)$  is exactly of class  $C^{m-2}$ . Thus the  $\Lambda(x)$  of (20) has exactly  $n - 2$  continuous derivatives.

2. It is expected that Polya frequency functions will be useful in descriptive statistics for the purpose of curve-fitting to empirical statistical data. One of the reasons for expecting such use is the extreme smoothness of these curves as a whole. By this we mean the following property: Let  $\Lambda(x)$  be a Polya frequency function of class  $C^{\infty}$ . By a repeated application of Rolle's theorem we conclude that  $\Lambda^{(n)}(x)$  has *at least*  $n$  distinct real zeros. Actually  $\Lambda^{(n)}(x)$  has *exactly*  $n$  simple real zeros and this is true for all values of  $n$ . An approach to Polya frequency functions from an entirely different

point of view will be discussed in a joint paper of H. B. Curry and the author.

3. A proof of Theorem 1 is essentially based on the results and methods developed by Polya and Schur. The only additional element required is a set of sufficient conditions insuring that a linear transformation be variation-diminishing.<sup>8</sup> Such conditions will serve to establish the following essential lemma: *If  $\Lambda(x)$  is a Polya frequency function and  $t_1 < t_2 < \dots < t_n$ , then the linear combination*

$$F(x) = a_1\Lambda(x - t_1) + a_2\Lambda(x - t_2) + \dots + a_n\Lambda(x - t_n)$$

*obeys the rule of signs of Descartes, i.e., the number of variations of sign of  $F(x)$ , for all real  $x$ , cannot exceed the number of variations of signs in the sequence  $a_1, a_2, \dots, a_n$ , of its coefficients.*

4. The discreet analogue of a t. p. function is a totally positive sequence  $\{a_n\}$  ( $n = 0, \pm 1, \pm 2, \dots$ ) with the property that the 4-way infinite matrix  $\|a_{i-j}\|$  has only non-negative minors. The author expects to discuss the nature and properties of such sequences on a future occasion.

<sup>1</sup> Determinants related to the one appearing here were considered by A. Bloch and G. Polya, "Abschätzungen des Betrages einer Determinante," *Vierteljahrsschrift Zürich*, **78**, 27-33 (1933).

<sup>2</sup> Polya, G., and Szegő, G., *Aufgaben und Lehrsätze aus der Analysis*, vol. 2, Problem 76, p. 49.

<sup>3</sup> Polya, G., and Schur, I., "Über zwei Arten von Faktorenfolgen in der Theorie der algebraischen Gleichungen," *Journal für Math.*, **144**, 89-113 (1914), especially p. 93.

<sup>4</sup> Polya, G., "Über Annäherung durch Polynome mit lauter reellen Wurzeln," *Rendiconti di Palermo*, **36**, 1-17 (1913). Laguerre assumes uniform convergence in every finite domain. Polya assumes such convergence only in a neighborhood of the origin.

<sup>5</sup> Polya, G., "Algebraische Untersuchungen über ganze Funktionen vom Geschlechte Null und Eins," *Journal für Math.*, **145**, 224-249 (1915).

<sup>6</sup> Hamburger, H., "Bemerkungen zu einer Fragestellung des Herrn Polya," *Math. Z.*, **7**, 302-322 (1920).

<sup>7</sup> We are actually using here the following addition to Theorem 1: If the reciprocal of a function of type II is represented by a Laplace integral (12), where  $\Lambda(x)$  is continuous, then  $\Lambda(x)$  is totally positive.

<sup>8</sup> Schoenberg, I., "Über variationsvermindernde lineare Transformationen," *Math. Z.*, **32**, 321-328 (1930), especially Satz 1.

# ON THE LOCATION OF THE CRITICAL POINTS OF HARMONIC MEASURE

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The object of this note is the proof of

**THEOREM 1.** *Let  $C$  be the unit circle in the  $z$ -plane, and let closed arcs  $\alpha_k$  ( $k = 1, 2, \dots, n$ ) of  $C$  be mutually disjoint. Denote by  $z = a_k$  and  $z = b_k$  the initial and terminal points of  $\alpha_k$ , with notation so chosen that the points  $a_k$  and  $b_k$  are ordered positively (i.e., counter-clockwise) on  $C$  as  $a_1, b_1, a_2, b_2, \dots, a_n, b_n, a_1 = a_{n+1}, b_1 = b_{n+1}$ . Denote by  $u(z)$  the harmonic measure with respect to the interior of  $C$  of the set  $\alpha = \alpha_1 + \alpha_2 + \dots + \alpha_n$  at a point  $z$  interior to  $C$ ; thus  $u(z)$  is harmonic and bounded interior to  $C$ , and approaches unity as  $z$  approaches an interior point of an arc  $\alpha_k$ , and approaches zero as  $z$  approaches an interior point of the arcs complementary to the set  $\alpha$ . Then no critical points of  $u(z)$  lie in the region  $R_k$  [or  $S_k$ ] interior to  $C$  bounded by the arc  $A_k: a_k b_k a_{k+1}$  [or  $B_k: b_k a_{k+1} b_{k+1}$ ] of  $C$  and the arc  $A'_k: a_k a_{k+1}$  [or  $B'_k: b_k b_{k+1}$ ] of a circle orthogonal to  $C$ . No critical point of  $u(z)$  lies on  $A'_k$  [or  $B'_k$ ] unless  $n = 2$ , in which case the unique critical point of  $u(z)$  lies at the intersection of  $A'_1$  and  $B'_1$ .*

Thus a point  $z$  interior to  $C$  cannot be a critical point of  $u(z)$  if a circle orthogonal to  $C$  separates  $z$  and one of the points  $a_k, b_k$  from all the other points  $a_j, b_j$ .

In the case  $n > 2$ , Theorem 1 enables us readily to construct a polygon of  $2n$  sides interior to  $C$  bounded by arcs of non-euclidean straight lines which contains in its interior all the  $n-1$  critical points of  $u(z)$  interior to  $C$ .

It is to be expected that the arcs  $A'_k$  and  $B'_k$  should play similar rôles in Theorem 1, for the harmonic measure with respect to the interior of  $C$  of the complement of the set  $\alpha$  in the point  $z$  is  $1 - u(z)$ , which has the same critical points as  $u(z)$ . In the proof of Theorem 1, we restrict ourselves to the case of  $R_k$  and the arcs  $A_k$  and  $A'_k$ , as is sufficient.

The harmonic measure of the arc  $\alpha_k$  with respect to  $C$  in the point  $z$  is readily computed to be

$$\frac{1}{\pi} [\arg(z - b_k) - \arg(z - a_k) - (1/2)\alpha_k], \quad (1)$$

where the arguments are suitably chosen, and where  $\alpha_k$  represents the angular measure of the arc  $\alpha_k$ . The function (1) is the real part of the analytic function

$$\frac{1}{\pi i} \left[ \log(z - b_k) - \log(z - a_k) - \frac{i\alpha_k}{2} \right]. \quad (2)$$

The critical points of  $u(z)$  are then the zeros of the function  $\varphi'(z)$ , where  $\varphi(z)$  is the sum of the functions (2) for all  $k$ ; we have

$$i\varphi'(z) = \frac{1}{\pi} \sum_{k=1}^n \left[ \frac{1}{z - b_k} - \frac{1}{z - a_k} \right]. \quad (3)$$

In (3) we take the conjugate of each term. The quantity  $1/(\bar{z} - \bar{b}_k)$  represents a vector of magnitude  $1/|z - b_k|$  whose direction and sense are those of the vector  $(z - b_k)$ . It follows that *the zeros of  $\varphi'(z)$  are the positions of equilibrium in the field of force due to unit positive particles situated at each point  $b_k$ , and unit negative particles situated at each point  $a_k$ , where each positive particle repels and each negative particle attracts, with a force equal to the inverse distance.*

The total force at the center 0 of  $C$  can be interpreted in magnitude, direction and sense as the sum of all the vectors  $b_k 0$  and  $0a_k$ , or as the sum of all the vectors  $b_k a_k$ . Moreover, in any conformal map of the interior of  $C$  onto itself the function  $u(z)$  is invariant, as are the critical points of  $u(z)$ , so we proceed to study those critical points by transforming  $C$  into itself so that the point to be studied is transformed into 0.

Let  $z_0$  denote an arbitrary point interior to  $R_k$  or on the arc  $A_k'$ ; we transform  $z_0$  into 0,  $1/\bar{z}_0$  into the point at infinity, and  $b_k$  into the point  $z = 1$ , by a linear transformation of  $z$ ; we retain the original notation. The point  $z_0 = 0$  lies in  $R_k$  or on  $A_k'$  so the arc  $A_k$  has angular measure at least  $\pi$ , and has angular measure  $\pi$  if and only if  $z_0$  lies on  $A_k'$ . The positive arc  $(a_{k+1}, -1)$  is not greater than the positive arc  $(a_k, +1)$ .

If  $z_0 = 0$  is a position of equilibrium in the field of force previously considered, the algebraic sum of the vectors  $b_j a_j$  must vanish. However, the point  $a_{k+1}$  cannot lie interior to the positive arc  $(b_k, -a_k)$ , and the points  $b_{k+1}, a_{k+2}, \dots, b_n, a_1, \dots, b_{k-1}$  lie on the positive arc  $a_{k+1}a_k$ , from which it follows that the sum of the horizontal components in the positive horizontal direction of the vectors  $b_{k+1}a_{k+1}, b_{k+2}a_{k+2}, \dots, b_n a_n, b_1 a_1, \dots, b_{k-1}a_{k-1}$  is algebraically less than the magnitude of the horizontal component of the vector  $b_k a_k$  (which is negative), except in the special case  $n = 2, a_1 = -a_2, b_1 = -b_2$ . Thus the total force at  $z_0 = 0$  cannot be zero, and  $z_0 = 0$  cannot be a critical point of  $u(z)$  except in this special case, and the theorem is established.

Theorem 1 is remarkable in the fact that for  $n > 2$  it exhibits a polygonal region containing all critical points, and yet a degenerate case of the region is the unique critical point in a non-trivial situation ( $n = 2$ ).

Theorem 1 can be interpreted as referring to the zeros of the derivative (or logarithmic derivative) of an arbitrary rational function

$$\prod_{k=1}^n \left( \frac{z - b_k}{z - a_k} \right)$$



whose poles and zeros are all simple and finite, and which alternate (i.e., are interlaced) on an arbitrary circle  $C$  of the extended plane. From this standpoint, the finite zeros of the derivative lie in two curvilinear polygons; the polygons lie one in each of the two regions into which  $C$  separates the plane; polygons and finite zeros are symmetric in  $C$ . The point at infinity is also a zero of the derivative.

Theorem 1 can obviously be extended by conformal mapping:

**THEOREM 2.** *Let  $C$  be an arbitrary Jordan curve, and let closed arcs  $\alpha_k$  ( $k = 1, 2, \dots, n$ ) of  $C$  be mutually disjoint. We denote by  $a_k$  and  $b_k$  the initial and terminal points of  $\alpha_k$ , with notation so chosen that the points  $a_k$  and  $b_k$  are ordered counter-clockwise on  $C$  as  $a_1, b_1, a_2, b_2, \dots, a_n, b_n, a_1 = a_{n+1}, b_1 = b_{n+1}$ . Denote by  $u(z)$  the harmonic measure with respect to the interior of  $C$  of the set  $\alpha_1 + \alpha_2 + \dots + \alpha_n$  at a point  $z$  interior to  $C$ . Then no critical points of  $u(z)$  lie in the region  $R_k$  [or  $S_k$ ] bounded by the arc  $A_k: a_k b_k a_{k+1}$  [or  $B_k: b_k a_{k+1} b_{k+1}$ ] of  $C$  and the arc  $A_k': a_k a_{k+1}$  [or  $B_k': b_k b_{k+1}$ ] of a non-euclidean straight line for the interior of  $C$ . No critical point of  $u(z)$  lies on  $A_k'$  unless  $n = 2$ , in which case the unique critical point of  $u(z)$  lies at the intersection of  $A_1'$  and  $B_1'$ .*

It may be noted that in Theorems 1 and 2 the arc  $A_k'$  [or  $B_k'$ ] is the locus of points  $z$  at which the harmonic measure of the arc  $A_k$  [or  $B_k$ ] of  $C$  with respect to the interior of  $C$  has the value one-half. Thus the conclusion of Theorems 1 and 2 may be expressed by asserting that at a critical point  $z$  of  $u(z)$  interior to  $C$ , the harmonic measure of every arc  $A_k$  [or  $B_k$ ] with respect to the interior of  $C$  has a value less than one-half, except in the case  $n = 2$ , when this value equals one-half.

Theorem 1 extends to the case of an infinite number of arcs  $\alpha_k$  and as thus extended applies in the study of critical points of harmonic measures in multiply connected regions, by a conformal map of their universal covering surfaces.

## THEORY OF HARMONIC TRANSFORMATIONS\*

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1. Consider a transformation  $T$  of the plane

$$X = \phi(x, y), \quad Y = \psi(x, y), \quad (1)$$

with the Jacobian  $J = \phi_x \psi_y - \phi_y \psi_x \neq 0$ , such that the components satisfy the Laplace equation

$$\phi_{xx} + \phi_{yy} = 0, \psi_{xx} + \psi_{yy} = 0. \quad (2)$$

We shall term any such correspondence  $T$  a *harmonic transformation*.

Harmonic correspondences should not be confused with conformal maps. In general, the components of a harmonic transformation are not interrelated in any way whatsoever. The components  $\phi$  and  $\psi$  of a conformal correspondence are of course conjugate-harmonic, that is, they satisfy the direct or reverse Cauchy-Riemann equations

$$\phi_x = \pm\psi_y, \phi_y = \mp\psi_x. \quad (3)$$

Thus a harmonic transformation is conformal if and only if its components are conjugate-harmonic.

All harmonic correspondences form an infinite set  $(H)$  of  $\infty^{4f(1)}$  transformations since they are defined by essentially four independent functions of a single variable. Our infinite set  $(H)$  contains as a subgroup the  $\infty^{2f(1)}$  conformalities which are defined by two independent functions of a single variable. The totality  $(H)$  of harmonic transformations, of course, do not constitute a group.

General harmonic transformations are of interest in connection with the theory of minimal surfaces and the Plateau problem. See the fundamental papers of Schwarz and Douglas. See footnote (5) for references.

We shall develop the theory of harmonic transformations from four points of view. See footnotes 1, 2 and 3. Firstly, we shall obtain various characterizations of the infinite set  $(H)$  of harmonic transformations by means of isothermal properties. From this, we deduce new characterizations of the conformal group. Secondly, we shall consider the groups contained in the infinite set  $(H)$ . Thirdly, we obtain various properties of  $(H)$  by means of the derivatives of a polygenic function. Fourthly, and finally, we shall give a discussion of the transformation theory of differential elements of first, second, and third orders (at a fixed point) induced by the infinite set  $(H)$ .

2. *If by a transformation  $T$  from the  $(x, y)$ -plane to the  $(X, Y)$ -plane, more than four distinct parallel pencils of straight lines in the  $(X, Y)$ -plane correspond to isothermal families in the  $(x, y)$ -plane, then in the real domain, either  $T$  is a harmonic transformation; or else  $T$  is the product of a conformality by a circle-to-line transformation. In all cases every parallel pencil of straight lines in the  $(X, Y)$ -plane corresponds to an isothermal family in the  $(x, y)$ -plane.*

If the restriction of the pencils of straight lines being parallel is removed, we obtain the following result.

*If by a transformation  $T$  from the  $(x, y)$ -plane to the  $(X, Y)$ -plane, every pencil (parallel or not) of straight lines in the  $(X, Y)$ -plane corresponds to an isothermal family in the  $(x, y)$ -plane, then  $T$  is either a conformal transforma-*

tion, or a circle-to-line correspondence, or the product of a conformality by a circle-to-line transformation.

3. By considering not only parallel pencils of straight lines but also concentric sets of circles, we derive the proposition:

*The only transformations in the real domain whereby every parallel pencil of straight lines and also every concentric set of circles correspond to isothermal families of curves are the conformalities.*

For the imaginary domain, the corresponding proposition is the following one.

*The only transformations in the real or imaginary domain whereby every pencil of circles corresponds to an isothermal family are the conformal maps.*

4. In general, the inverse of a harmonic transformation is not harmonic.<sup>6</sup> We obtain all harmonic correspondences with harmonic inverses. The following result is established.

*In the real domain, the complete set of harmonic transformations whose inverses are also harmonic consists of those of the infinite conformal group, those of the six-parameter affine group, and those of the eight-parameter set of transformations*

$$mU = \log \frac{ae^{nu} + b}{A + Be^{-Nv}}, \quad MV = \log \frac{Ae^{Nv} + B}{a + be^{-nu}}, \quad (4)$$

where  $(A, B, M, N)$  are the conjugates of the constants  $(a, b, m, n)$ , and where  $m \neq 0, M \neq 0, n \neq 0, N \neq 0$ , and  $aA - bB \neq 0$ .

Here  $(u, v)$  denote minimal coordinates of a point, that is,  $u = x + iy$  and  $v = x - iy$ .

The inverse of any transformation of the eight-parameter set (4) is in the set. If  $S(a, b, m, n)$  is any transformation of this set, its inverse is  $S(A, -b, n, m)$ . The identity is given by  $a = A, m = n, b = 0$ .

However this set (4) does not constitute a group since the product of any two transformations of this set (4) is not in the set (4) in general.

In the imaginary domain, it is found that there are four other sets of harmonic transformations with harmonic inverses in addition to the three sets stated above.

5. *In the imaginary domain, the only groups of harmonic transformations are the infinite conformal group, the six-parameter affine group, the two infinite groups*

$$\begin{aligned} U &= au + g(v), \quad V = F(v), \quad a \neq 0, \quad F_v \neq 0; \\ U &= f(u), \quad V = av + G(u), \quad a \neq 0, \quad f_u \neq 0; \end{aligned} \quad (5)$$

and the subgroups of these four groups.

In the real domain, the only harmonic groups are the conformal group, the affine group, and the subgroups of these two.

6. *A point transformation  $T$  is harmonic if and only if the center trans-*

formation induced by the first derivative of the related polygenic function  $w = \phi + i\psi$  is direct conformal.

We find other properties of harmonic transformations with reference to the second derivative of the polygenic function. One such result is the following.

*If a transformation  $T$  is harmonic, then the fundamental conic section  $Q$  is a horizontal parabola<sup>4</sup>.*

7. We consider the transformation theory of differential elements of first, second and third orders (at a fixed point) induced by the infinite set (II) of harmonic transformations. For the cases of first and second order differential elements, this transformation theory for the harmonic case is identical with that induced by the total group of all point transformations. But for third order differential elements, we establish the following proposition.

*The infinite set (II) of harmonic transformations induces on the differential elements of third order a twelve-parameter set  $S_{12}$  of transformations, which is a subset of the fifteen-parameter group  $G_{15}$  induced by the group of all-point transformations.*

Elsewhere we shall develop a characterization of this twelve-parameter set of transformations  $S_{12}$  of third order differential elements.

\* Presented to the American Mathematical Society, 1946.

The following papers constitute a short bibliography of the related subjects developed by Kasner and jointly by Kasner and De Cicco.

1. "The Geometry of Differential Elements of Second Order with Respect to the Group of All Point Transformations," *Amer. Journ. Math.*, **28**, 203-213 (1906).

2. "Biharmonic Functions and Certain Generalizations," *Amer. Journ. Math.*, **58**, 377-390 (1936).

3. "The Pseudo-Angle in Space of  $2n$ -Dimensions"; also "Bi-Isothermal Systems," *Bull. Amer. Math. Soc.*, **51**, 162-174 (1945). "Circle-to-line Transformations," *Amer. Math. Monthly*, **52**, 425-433, (1945).

4. "The Geometry of Polygenic Functions," *Revista Universidad Tucuman, Argentina*, **4**, 7-45 (1944). "The Second Derivative of a Polygenic Function," *Trans. Amer. Math. Soc.* **30**, 803-818 (1928).

5. Schwarz, *Abhandlungen*, Vol. 1, p. 293. Also see abstracts by Douglas in *Bull. Amer. Math. Soc.* (1943).

6. Douglas also has studied the inversion of harmonic transformations. See *Bull. Amer. Math. Soc.* (1944), p. 180.





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## COMPLETE CONVERGENCE AND THE LAW OF LARGE NUMBERS

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1. We begin by listing some standard definitions in the theory of probability. A *probability space* is a set  $\Omega$  of elements  $\omega$  together with a  $\sigma$ -field  $m$  of subsets of  $\Omega$  on which is defined a completely additive measure  $P$  such that  $P(\Omega) = 1$ . A real-valued  $P$ -measurable function  $X = X(\omega)$  is a *random variable*, and the function  $F(x) = P\{X \leq x\}$ , where  $\{\cdot\}$  denotes the set of all  $\omega$  such that the relation within the braces holds, is the *distribution function* of  $X$ . The sets of a sequence  $A_1, A_2, \dots$  are *independent* if for every finite set  $i_1, \dots, i_n$  of distinct integers,  $P(\prod_{r=1}^n A_{i_r}) = \prod_{r=1}^n P(A_{i_r})$ , and the random variables of a sequence  $X_1, X_2, \dots$  are independent if, for every sequence  $x_1, x_2, \dots$  of real numbers, the sets  $\{X_1 \leq x_1\}, \{X_2 \leq x_2\}, \dots$  are independent.

For purposes of comparison we list the following modes in which a sequence

$$X_1, X_2, \dots \tag{1}$$

of random variables defined on  $\Omega$  may converge to 0.

(i) The sequence (1) converges to 0 *in probability* if for every  $\epsilon > 0$ ,

$$\lim_{n \rightarrow \infty} P\{|X_n| > \epsilon\} = 0.$$

(ii) The sequence (1) converges to 0 *with probability 1* if for every  $\epsilon > 0$ ,

$$\lim_{n \rightarrow \infty} P\{\{|X_n| > \epsilon\} + \{|X_{n+1}| > \epsilon\} + \dots\} = 0.$$

It is easily seen that this is equivalent to the usual condition,  $P\{\lim_{n \rightarrow \infty} X_n = 0\} = 1$ , and that (ii) implies (i) but not conversely.

2. We shall be concerned with a third mode of convergence, which, for want of a better name, we call *complete*.

(iii) The sequence (1) converges to 0 *completely* if for every  $\epsilon > 0$ ,

$$\lim_{n \rightarrow \infty} [P\{|X_n| > \epsilon\} + P\{|X_{n+1}| > \epsilon\} + \dots] = 0.$$

Clearly, (iii) implies (ii). The example:  $\Omega =$  unit interval  $0 < \omega < 1$ ,  $P =$  Lebesgue measure,  $X_n = 1$  for  $0 < \omega < \frac{1}{n}$  and 0 otherwise, shows that (ii) does not imply (iii).

Let us call two sequences of random variables  $X_1, X_2, \dots$  and  $Y_1, Y_2, \dots$ , defined, respectively, on probability spaces  $\Omega$  and  $\Omega_1$ , *F-equivalent*, if for every  $n$  the distribution function of  $Y_n$  is identical with that of  $X_n$ . Definitions (i) and (iii) are invariant under *F*-equivalence, while (ii) is not. However, a sequence  $X_1, X_2, \dots$  of random variables converges to 0 *completely* if and only if every *F*-equivalent sequence converges to 0 with probability 1. The necessity is obvious; to prove sufficiently consider a sequence  $Y_1, Y_2, \dots$  of independent random variables *F*-equivalent to the given sequence. If the sequence  $Y_1, Y_2, \dots$  converges to 0 with probability 1 then for any  $\epsilon > 0$ ,  $P\{\limsup_{n \rightarrow \infty} \{|Y_n| > \epsilon\}\} = 0$ . Since the sets  $\{|Y_n| > \epsilon\}$  are independent, it follows from a theorem of Borel-Cantelli<sup>1</sup> that  $\sum_{n=1}^{\infty} P\{|Y_n| > \epsilon\} = \sum_{n=1}^{\infty} P\{|X_n| > \epsilon\} < \infty$ .

It follows from this proof that if  $X_1, X_2, \dots$  is a sequence of *independent* random variables, then definitions (ii) and (iii) are equivalent.

3. Let the random variables  $X_n$  in (1) be independent with the same distribution function  $F(x) = P\{X_n \leq x\}$  and such that the expectation  $E(X_n) = \int_{-\infty}^{\infty} x dF(x) = 0$ . The *strong law of large numbers* for identically distributed random variables states that the sequence of random variables  $Y_1, Y_2, \dots$ , where for each  $n$

$$Y_n = (X_1 + \dots + X_n)/n \quad (2)$$

converges to 0 with probability 1. We shall show in Theorems 1 and 2 that under the same hypotheses the sequence (2) need not converge to 0 completely, but that it will do so under the further hypothesis that  $\int_{-\infty}^{\infty} x^2 dF(x) < \infty$ .

4. THEOREM 1. Let (1) be a sequence of independent random variables with the same distribution function  $F(x)$  and such that

$$\int_{-\infty}^{\infty} x dF(x) = 0, \quad \sigma^2 = \int_{-\infty}^{\infty} x^2 dF(x) < \infty. \quad (3)$$

Then the sequence (2) converges to 0 completely; i.e., the series

$$\sum_{n=1}^{\infty} P\{|Y_n| > \epsilon\} \quad (4)$$

converges for every  $\epsilon > 0$ .

*Proof.* We shall prove the theorem for  $\epsilon = 2$ . This is no restriction since we can always consider  $\frac{2}{\epsilon}X_n$  instead of  $X_n$ . Moreover, we may assume that  $\sigma^2 > 0$ .

Let  $f(t) = \int_{-\infty}^{\infty} e^{itx} dF(x)$  be the characteristic function of the distribution  $F(x)$ . From (3) it follows that constants  $\alpha, \alpha', \alpha''$  exist such that

$$|1 - f(t)| \leq \alpha t^2, \quad |f'(t)| \leq \alpha' t, \quad |f''(t)| \leq \alpha''. \quad (5)$$

Choose and fix a positive  $\delta$  so small that for  $|t| \leq 4\delta$  the following conditions (6) and (7) are satisfied:

$$|\sin^{1/2} t| \geq Bt, \quad |(1 - f(t))^2 - 4(1 - f(t)) \sin^2 1/2 t + 4 \sin^2 1/2 t| \geq Ct^2, \quad (6)$$

where  $B$  and  $C$  are constants,

$$|f(t)| \neq 1 \quad \text{except at } t = 0. \quad (7)$$

Let  $Z$  be a random variable distributed with the density  $3(2\pi)^{-1}x^{-4} \sin^4 x$  and hence the characteristic function

$$\varphi(t) = \begin{cases} 1 - 3/8t^2 + 3/32|t|^3, & |t| \leq 2, \\ 1/32(4 - |t|)^2, & 2 \leq |t| \leq 4, \\ 0 & 4 \leq |t|. \end{cases} \quad (8)$$

We regard  $Z$  as independent of  $Y_n$  and use addition in this sense. Since

$$\begin{aligned} P\{|Y_n| > 2\} &\leq P\left\{\left|Y_n + \frac{Z}{n\delta}\right| > 1\right\} + P\left\{\left|\frac{Z}{n\delta}\right| > 1\right\} = \\ &P\left\{\left|\frac{Z}{n\delta}\right| \leq 1\right\} - P\left\{\left|Y_n + \frac{Z}{n\delta}\right| \leq 1\right\} + 2P\left\{\left|\frac{Z}{n\delta}\right| > 1\right\}, \end{aligned}$$

and since

$$\sum_{n=1}^{\infty} P\left\{\left|\frac{Z}{n\delta}\right| > 1\right\} \leq \frac{3}{\pi} \sum_{n=1}^{\infty} \int_{n\delta}^{\infty} \frac{dx}{x^4} = \frac{1}{\pi\delta^3} \sum_{n=1}^{\infty} \frac{1}{n^3} < \infty,$$

it is sufficient to prove that

$$\sum_{n=1}^{N-1} \left[ P\left\{\left|\frac{Z}{n\delta}\right| \leq 1\right\} - P\left\{\left|Y_n + \frac{Z}{n\delta}\right| \leq 1\right\} \right] = o(1), \quad (9)$$

where  $o(1)$  always denotes a quantity bounded with respect to  $N$ .

The characteristic function  $f^n\left(\frac{t}{n}\right) \varphi\left(\frac{t}{n\delta}\right)$  of  $Y_n + \frac{Z}{n\delta}$  vanishes for  $|t| > 4n\delta$ ; hence by a well-known inversion formula,<sup>2</sup>

$$P\left\{\left|Y_n + \frac{Z}{n\delta}\right| \leq 1\right\} = \frac{1}{\pi} \int_{-4n\delta}^{4n\delta} f^n\left(\frac{t}{n}\right) \varphi\left(\frac{t}{n\delta}\right) \frac{\sin t}{t} dt =$$

$$\frac{1}{\pi} \int_{-4\delta}^{4\delta} f^n(t) \varphi\left(\frac{t}{\delta}\right) \frac{\sin nt}{t} dt.$$

Also,

$$P\left\{\left|\frac{Z}{n\delta}\right| \leq 1\right\} = \frac{1}{\pi} \int_{-4\delta}^{4\delta} \varphi\left(\frac{t}{\delta}\right) \frac{\sin nt}{t} dt.$$

Hence, by subtraction the left side of (9) is equal to

$$\frac{1}{\pi} \int_{-4\delta}^{4\delta} \frac{1}{t} \varphi\left(\frac{t}{\delta}\right) \sum_{n=1}^{N-1} (1 - f^n(t)) \sin nt dt = \frac{1}{\pi} A_N, \text{ say.} \quad (10)$$

From now on we write  $f$  for  $f(t)$ . Direct computation gives the result

$$\sum_{n=1}^{N-1} (1 - f^n) \sin nt = \frac{(1 - f)^2 \sin \frac{1}{2}Nt \sin \frac{1}{2}(N-1)t}{q(t) \sin \frac{1}{2}t}$$

$$+ \frac{(1 - f) \sin t}{q(t)} - \frac{4(1 - f) \sin \frac{1}{2}t \sin \frac{1}{2}Nt \sin \frac{1}{2}(N-1)t}{q(t)}$$

$$+ \frac{(1 - f)f^N \sin Nt}{q(t)} - \frac{2(1 - f^{N+1}) \sin \frac{1}{2}t \cos (N - \frac{1}{2})t}{q(t)}, \quad (11)$$

where

$$q(t) = (f - e^{it})(f - e^{-it}) = (1 - f)^2 - 4(1 - f) \sin^2 \frac{1}{2}t + 4 \sin^2 \frac{1}{2}t. \quad (12)$$

By (6) we have  $|q(t)| \geq Ct^2$ ,  $|q(t) \sin \frac{1}{2}t| \geq C'|t|^3$ , where  $C$  and  $C'$  are constants. Hence when (11) is substituted into (10) and the first inequality of (5) is used, we see that the first three terms merely contribute  $O(1)$ . Consequently,

$$A_N = \int_{-4\delta}^{4\delta} \varphi\left(\frac{t}{\delta}\right) \frac{(1 - f)f^N \sin Nt - 2(1 - f^{N+1}) \sin \frac{1}{2}t \cos (N - \frac{1}{2})t}{tq(t)} dt$$

$$+ O(1). \quad (13)$$

For  $\delta \leq |t| \leq 4\delta$  we have, by (7),  $|f| \neq 1$ , hence  $q(t) = |f - e^{it}|^2 \geq (1 - |f|)^2 \geq a > 0$ . Therefore the part of the integral in (13) extended over the range  $\delta \leq |t| \leq 4\delta$  is  $O(1)$ , so that (13) holds with  $4\delta$  replaced by  $\delta$  in the two limits of integration. For  $|t| \leq \delta$ , however,  $\varphi\left(\frac{t}{\delta}\right) = 1 - \frac{3t^2}{8\delta^2} + \frac{3|t|^3}{32\delta^3}$ , and the terms with  $t^2$  and  $|t|^3$  are easily seen to contribute  $O(1)$ . Hence,

$$A_N = \int_{-\delta}^{\delta} \frac{(1-f)f^N \sin Nt - 2(1-f^{N+1}) \sin^{1/2}t \cos(N-1/2)t}{tq(t)} dt + 0(1). \quad (14)$$

Since

$$\left| \frac{1}{tq(t)} - \frac{1}{t^3} \right| = \left| \frac{t^2 - q(t)}{t^3q(t)} \right| \leq k \frac{t^4}{|t|^5} = \frac{k}{|t|},$$

where  $k$  is a constant, the replacement of  $tq(t)$  by  $t^3$  in (14) will make a difference of only  $0(1)$ , so that

$$A_N = \int_{-\delta}^{\delta} \frac{(1-f)f^N \sin Nt}{t^3} dt - \int_{-\delta}^{\delta} \frac{2(1-f^{N+1}) \sin^{1/2}t \cos(N-1/2)t}{t^3} dt + 0(1). \quad (15)$$

Let the two integrals in (15) be denoted by  $I_N$  and  $J_N$ , respectively. We have

$$I_N = \frac{2}{N} \int_{-\delta}^{\delta} \frac{(1-f)f^N}{t^3} d(\sin^2 1/2 Nt) = 0(1) + \frac{2}{N} \int_{-\delta}^{\delta} \left\{ \frac{3(1-f)f^N}{t^4} + \frac{f^N f'}{t^3} - \frac{Nf^{N-1}(1-f)f'}{t^3} \right\} \sin^2 1/2 Nt dt.$$

Using the first two inequalities of (5) we obtain the result

$$|I_N| \leq 0(1) + (6\alpha + 2\alpha') \int_{-\infty}^{\infty} \frac{\sin^2 1/2 Nt}{Nt^2} dt + 2 \int_{-\infty}^{\infty} \frac{|1-f| |f'|}{|t^3|} dt = 0(1),$$

since the integral involving  $N$  is independent of  $N$ .

To deal with  $J_N$  we observe first that in  $J_N$ ,  $\sin^{1/2}t$  may be replaced by  $1/2t$  and  $f^{N+1}$  by  $f^N$ , the difference thus made being  $0(1)$ . Hence

$$J_N = \int_{-\delta}^{\delta} \frac{(1-f^N) \cos(N-1/2)t}{t^2} dt + 0(1) = \int_{-\infty}^{\infty} \frac{(1-f^N) \cos(N-1/2)t}{t^2} dt + 0(1).$$

We may replace  $\cos(N-1/2)t$  by  $\cos Nt$ , since

$$\begin{aligned} \frac{2}{\pi} \int_{-\infty}^{\infty} \frac{1-f^N}{t^2} (\cos(N-1/2)t - \cos Nt) dt &= \frac{1}{\pi} \int_{-\infty}^{\infty} (1-f^N) \frac{\sin^{1/4}t}{1/4t} \\ &\quad \frac{\sin(N-1/4)t}{t} dt = P\{|U| \leq N-1/4\} - P\{|U+NY_N| \leq N-1/4\} \\ &= 0(1), \end{aligned}$$



where  $U$  is a random variable independent of  $Y_N$  and whose characteristic function is  $4/t \sin^{1/2} t$ . Hence

$$\begin{aligned} J_N &= 0(1) + \frac{1}{N} \int_{-\infty}^{\infty} \frac{1 - f^N}{t^2} d \sin Nt = 0(1) + \frac{2}{N^2} \int_{-\infty}^{\infty} \left\{ \frac{2(1 - f^N)}{t^3} \right. \\ &\quad \left. + \frac{N f^{N-1} f'}{t^2} \right\} d \sin^{1/2} t \\ &= 0(1) + \frac{2}{N^2} \int_{-\infty}^{\infty} \left\{ \frac{6(1 - f^N)}{t^4} + \frac{4N f^{N-1} f'}{t^3} - \frac{N(N-1) f^{N-2} f'^2}{t^2} \right. \\ &\quad \left. - \frac{N f^{N-1} f''}{t^2} \right\} \sin^{1/2} t dt. \end{aligned}$$

Using all the inequalities (5) we have

$$|J_N| \leq 0(1) + (12\alpha + 8\alpha' + 2\alpha'') \int_{-\infty}^{\infty} \frac{\sin^{1/2} t}{Nt^2} dt + 2 \int_{-\infty}^{\infty} \frac{|f'|^2}{t^2} dt = 0(1)$$

The proof is now complete.

5. By following the essential steps of the proof of Theorem 1 we obtain the following theorem, the proof of which is omitted from the present communication.

THEOREM 2. *If instead of conditions (3) we have*

$$\int_{-\infty}^{\infty} x dF(x) = 0, \int_{-\infty}^{\infty} |x|^a dF(x) < \infty, \int_{-\infty}^{\infty} x^2 dF(x) = \infty \quad (16)$$

where  $a$  is some constant such that  $\frac{1}{2}(1 + 5^{1/2}) \leq a < 2$ , then the series (4) diverges for every  $\epsilon > 0$ . (Example: Let  $X_n$  be distributed with the density  $|x|^{-3}$  for  $|x| \geq 1$  and 0 elsewhere.)

Since the finiteness of the second integral in (16) would seem rather to favor than to oppose the convergence of (4), it may be conjectured that given the first condition of (3), the finiteness of  $\sigma^2$  is not only sufficient but also necessary for the convergence of (4). We have not been able to prove this.

6. The following generalization<sup>3</sup> of the strong law of large numbers is an immediate consequence of Theorem 1 and the remarks in section 2.

THEOREM 3. *Let  $X_n^{(r)}$  ( $n = 1, 2, \dots$ ;  $r = 1, \dots, n$ ) be an array of random variables with the same distribution function  $F(x)$  and such that (1)*

*$\int_{-\infty}^{\infty} x dF(x) = 0$ ,  $\int_{-\infty}^{\infty} x^2 dF(x) < \infty$ , and (2) for each  $n$  the random variables  $X_1^{(n)}, \dots, X_n^{(n)}$  are independent. Then the sequence of random variables  $Y_1, Y_2, \dots$ , where for each  $n$ ,  $Y_n = (X_1^{(n)} + \dots + X_n^{(n)})/n$ , converges to 0 with*

probability 1. (Note that we do not assume any relation of dependence or independence between  $X_n^{(r)}$  and  $X_m^{(s)}$  for  $m \neq n$ .)

<sup>1</sup> See M. Fréchet, *Recherches théoriques modernes*, Vol. 1, Paris, 1937, p. 27.

<sup>2</sup> See H. Cramér, *Mathematical methods of statistics*, Princeton, 1946, p. 98.

<sup>3</sup> Compare F. P. Cantelli, Considerazioni sulla legge uniforme dei grandi numeri ecc., *Giornale dell'Istituto Italiano degli Attuari*, IV (1933), pp. 331-332; also H. Cramér, Su un theorema relativo alla leggi uniforme dei grandi numeri, *Ibid.*, V (1934), pp. 1-13.

## GREEN'S FUNCTIONS FOR LINEAR DIFFERENTIAL SYSTEMS OF INFINITE ORDER

BY D. V. WIDDER

In these PROCEEDINGS<sup>1</sup> the author sketched a theory whereby a special differential equation of infinite order

$$\frac{\sin \pi D}{\pi} y(x) = \varphi(x) \quad (1)$$

could be solved by use of a Green's function. The operator on the left of this equation is interpreted to mean

$$\lim_{n \rightarrow \infty} D \left(1 - \frac{D^2}{1^2}\right) \cdots \left(1 - \frac{D^2}{n^2}\right) y(x),$$

where  $D$  is the operation of differentiation with respect to  $x$ . In preparing the details of this theory the author discovered that much more general differential equations could be treated by the same method. It is the purpose of the present note to outline this more general theory.

We define an entire function  $E(s)$  as follows:

$$E(s) = s \prod_{k=1}^{\infty} \left(1 - \frac{s^2}{a_k^2}\right), \quad (2)$$

where the constants  $a_k$  are real and such that

$$0 < a_1 < a_2 < \dots \quad (3)$$

$$\sum_{k=1}^{\infty} \frac{1}{a_k^2} < \infty. \quad (4)$$

Consider now the differential system

$$E(D)y(x) = \varphi(x) \quad (5)$$

$$y(-\infty) = 0 \quad (6)$$

$$y(+\infty) = \int_{-\infty}^{\infty} \varphi(t) dt. \quad (7)$$

We are assuming that  $\varphi(x)$  is a given continuous function which is absolutely integrable on  $(-\infty, \infty)$ . In particular, if  $a_k = k$ , equation (5) reduces to equation (1).

It is natural to define the Green's function,  $G(x)$ , for the system (5) (6) (7) as the limit of the Green's function,  $G_{2n+1}(x)$ , of the "truncated" system

$$D \prod_{k=1}^n \left( 1 - \frac{D^2}{a_k^2} \right) y(x) = \varphi(x) \quad (8)$$

with boundary conditions (6) and (7). We recall the definition<sup>2</sup> of  $G_{2n+1}(x)$ . It satisfies the semihomogeneous system

$$D \prod_{k=1}^n \left( 1 - \frac{D^2}{a_k^2} \right) y(x) = 0$$

$$y(-\infty) = 0, y(+\infty) = 1$$

for all  $x$  different from zero. It is continuous with its first  $(2n-1)$  derivatives for all  $x$ . Its  $2n$ th derivative is continuous except at  $x = 0$ , where

$$y^{(2n)}(0+) - y^{(2n)}(0-) = (-1)^n a_1^2 a_2^2 \dots a_n^2.$$

It is easy to see that these conditions determine  $G_{2n+1}(x)$  uniquely and that the unique solution of the system (8) (6) (7) is

$$y(x) = \int_{-\infty}^{\infty} G_{2n+1}(x-t) \varphi(t) dt.$$

We can, in fact, write down an explicit integral formula for  $G_{2n+1}(x)$ :

$$G_{2n+1}(x) = \frac{1}{2\pi i} \int_{c-i\infty}^{c+i\infty} \frac{e^{sx}}{s \prod_{k=1}^n \left( 1 - \frac{s^2}{a_k^2} \right)} ds \quad 0 < c < a_1.$$

This integral can in turn be evaluated by use of the calculus of residues, and is two distinct linear combinations of exponential functions in the two intervals  $(-\infty, 0)$  and  $(0, \infty)$ .

We are next able to show that  $G_{2n+1}(x)$  tends to a limit  $G(x)$  as  $n$  becomes infinite, and that this function yields a solution

$$y(x) = \int_{-\infty}^{\infty} G(x-t) \varphi(t) dt \quad (9)$$

of the system (5) (6) (7). In the course of the proof of these facts, we show that the reciprocal of the function  $E(s)$  is a bilateral Laplace transform.

$$\frac{1}{E(s)} = \int_{-\infty}^{\infty} e^{-st} G(t) dt,$$

convergent in the interval  $0 < s < a_1$ . In fact the determining function, as we have indicated by the notation, is the Green's function described above.

We summarize the above results in the following theorem.

**THEOREM.** *If  $\varphi(x) \in C \cdot L$  in  $(-\infty < x < \infty)$ , if  $E(s)$  is an entire function defined by (2) (3) (4), and if*

$$G(x) = \frac{1}{2\pi i} \int_{c-i\infty}^{c+i\infty} \frac{e^{sx}}{E(s)} ds \quad 0 < c < a_1,$$

*then the function  $y(x)$  defined by (9) satisfies the system (5) (6) (7).*

We have thus obtained a real linear differential inversion formula for the general Faltung-equation (9). In particular, if  $a_k = k$ ,  $G(x)$  becomes

$$G(x) = \frac{1}{2i} \int_{c-i\infty}^{c+i\infty} \frac{e^{sx}}{\sin \pi s} ds = \frac{1}{1 + e^{-x}},$$

and equation (9) becomes

$$y(x) = \int_{-\infty}^{\infty} \frac{\varphi(t)e^{-t}}{e^{-t} + e^{-x}} dt,$$

or

$$f(x) = y\left(\log \frac{1}{x}\right) = \int_0^{\infty} \frac{\varphi\left(\log \frac{1}{t}\right)}{x+t} dt \quad (10)$$

after an exponential change of variable. The same exponential change of variable applied to the differential operator (1) shows that

$$\lim_{k \rightarrow \infty} \frac{(-1)^{k-1}}{k!(k-2)!} [x^{2k-1} f^{(k-1)}(x)]^k = \varphi\left(\log \frac{1}{x}\right).$$

We thus rediscover, as a special case of the present theory, the author's real inversion formula for the Stieltjes transform.<sup>3</sup> In the light of this general theory the original discovery of that formula seems somewhat fortuitous, depending as it did on the Laplace asymptotic evaluation of an integral of the form

$$\int_0^{\infty} [g(t)]^n \varphi(t) dt. \quad (11)$$

It was the special nature of the constants  $a_k$  as integers that introduced powers of a function  $g(t)$ . We are now able to retain the essential features of the method even though the integral is now replaced by another which no longer involves powers of a function. One of the outstanding features of the present theory is that it enables one to set up a whole new class of inversion formulas for integral transforms. One may either discover the

kernel of the transform from a given inversion operator, or, conversely, set up the inversion operator when the kernel is given. The present approach is related to the fundamental work of N. Wiener on the operational calculus.<sup>4</sup> It should be observed, however, that there is here no appeal to the  $L^2$ -Fourier transform theory.

<sup>1</sup> Widder, D. V., "The Green's Function for a Differential System of Infinite Order," *Proc. Nat. Acad. Sci.*, 26, 213-215 (1940).

<sup>2</sup> See, for example, M. Bôcher, *Leçons sur les méthodes de Sturm*, Paris (1917), Chap. V.

<sup>3</sup> Widder, D. V., "The Stieltjes Transform," *Trans. Am. Math. Soc.*, 43, 7-60 (1938).

<sup>4</sup> Wiener, N., "The Operational Calculus," *Math. Annalen*, 95, 557-584 (1926).

## SPECIAL VALUES OF $e^{k\pi}$ , $\cosh(k\pi)$ AND $\sinh(k\pi)$ TO 136 FIGURES

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The present investigation was undertaken with two primary objects in view, namely, to obtain certain heretofore uncomputed values of  $e^{k\pi}$ ,  $\cosh(k\pi)$  and  $\sinh(k\pi)$ , and to extend appreciably the degree of approximation of data previously found by others and by the author.

The actual calculations were based on his 137-place table of logarithms because preliminary tests of available infinite series showed that their application would have necessitated a much greater expenditure of labor and time than the radix logarithms. For example, the 79th term of the series  $e^z = \sum_{n=0}^{\infty} (z^n/n!)$ , when  $z = \pi/6$ , has 139 zeros between the decimal point and the leading figures 70673. Seventy-nine terms would not have been deterrent but for  $n > 2$  examination of page 84 of the invaluable *Index of Mathematical Tables*<sup>1</sup> revealed the facts that the published values of  $\pi^n$  required for the earlier terms of the series attained 41 figures in one dubious case and that for somewhat lower degrees of approximation only 32 consecutive entries were tabulated. Again, consider the series  $e^{\sin^{-1} x}$  which was chosen by the author in an earlier article<sup>2</sup> because  $x$  has the rational value  $1/2$  when  $\sin^{-1} x = \pi/6$ . The general expression for the terms involving even powers of  $x$  may be written

$$t_{2k+1} = (0^2 + 1)(2^2 + 1)(4^2 + 1) \dots [(2k - 2)^2 + 1]x^{2k}/(2k!).$$

When  $x = 1/2$  the course of this function of  $k$  and the prohibitively slow convergence of the series may be seen from the following values of  $t_{2k+1}$   
 $t_{43} = 5.0977 \times 10^{-28}$ ,  $t_{103} = 1.5209 \times 10^{-32}$ ,  $t_{243} = 6.8957 \times 10^{-37}$ ,  $t_{523} =$

$3.7173 \times 10^{-101}$ ,  $t_{403} = 1.7512 \times 10^{-126}$  and  $t_{461} = 6.6138 \times 10^{-140}$ , where  $k = 41, 81, 121, 161, 201$  and  $225$ , respectively.

A condensed account of the details of the performance of the calculations for table 1 will be presented now in order to emphasize the precautions taken by the author to reduce the probability of error to an extremely small value.

First  $e^{+\pi/2}$  was calculated from the original master strips upon which the 137-place printed table of negative radix logarithms had been based. All of these fundamental data extended well beyond the 137th place of decimals. The most error-sensitive part of the work consisted as usual in multiplying together mentally the binomial factors as obtained from the logarithms. Hence, this set of operations was performed three times. Next  $e^{-\pi/2}$  was computed from  $e^{+\pi/2}$  by machine multiplication reversed. The checking product  $e^{+\pi/2} \cdot e^{-\pi/2}$  gave 150 consecutive 9's. Then  $e^{+\pi}$  and  $e^{-\pi}$  were found by hand-machine squaring, respectively,  $e^{+\pi/2}$  and  $e^{-\pi/2}$ .  $e^{+\pi/2} \cdot e^{-\pi/2}$  was performed twice. The check  $e^{+\pi} \cdot e^{-\pi}$  yielded 150 successive 9's.

After this  $e^{-\pi/192}$  was computed from the master data of negative radix logarithms with the same extreme care as explained above. By repeated squaring the values of  $e^{-\pi/96}$ ,  $e^{-\pi/48}$ ,  $e^{-\pi/24}$ ,  $e^{-\pi/12}$ ,  $e^{-\pi/6}$  and  $e^{-\pi/3}$  were calculated. In this sequence, and for all machine multiplications referred to below, the act of multiplying was performed independently twice, first with the figures segregated as octads and second with nonad grouping. To check the work  $e^{-\pi/2}$  was obtained from  $e^{-\pi/3} \cdot e^{-\pi/6}$ . This approximation to  $e^{-\pi/2}$  was less than that derived at the beginning from a different set of logarithms by 3.547 units in the 140th decimal place. This means a per cent error of  $-1.706 \times 10^{-137}$  for the second determination of  $e^{-\pi/2}$  when the first value is taken as standard.

Diminishing in details, reciprocating  $e^{-\pi/192}$  gave  $e^{+\pi/192}$ . The product of these numbers produced 151 consecutive 9's. Squaring led to  $e^{+\pi/96}$ ,  $e^{+\pi/48}$ ,  $e^{+\pi/24}$ ,  $e^{+\pi/12}$ ,  $e^{+\pi/6}$  and  $e^{+\pi/3}$ . The product  $e^{+\pi/3} \cdot e^{+\pi/6}$  was greater than the value of  $e^{+\pi/2}$  as first found by 8.206 units in the 139th decimal place. This is equivalent to a per cent error of  $+1.706 \times 10^{-137}$ .  $e^{+\pi/192} \cdot e^{+\pi/96}$  gave  $e^{+\pi/64}$ . Successive squaring produced  $e^{+\pi/32}$ ,  $e^{+\pi/16}$ ,  $e^{+\pi/8}$ ,  $e^{+\pi/4}$  and  $e^{+\pi/2}$ . The "error" equaled  $+7.381$  units in the 139th decimal place or  $+1.534 \times 10^{-137}$  per cent.

Finally  $e^{-\pi/192} \cdot e^{-\pi/96}$  gave  $e^{-\pi/64}$ . Repeated squaring furnished  $e^{-\pi/32}$ ,  $e^{-\pi/16}$ ,  $e^{-\pi/8}$ ,  $e^{-\pi/4}$  and  $e^{-\pi/2}$ . The "error" was  $-3.546$  units in the 140th decimal place, that is  $-1.706 \times 10^{-137}$  per cent.

It should now be clear that the interval between the reciprocal pair  $(e^{+\pi/192}, e^{-\pi/192})$  and the pair  $(e^{+\pi/2}, e^{-\pi/2})$  had been bridged successfully by four different sequences so that the constants in table 1 are bound together as an unimpeachable unit. Nevertheless, as an extreme precaution



the product  $e^{-\pi/16} \cdot e^{+\pi/16}$  was formed and found to equal  $1 + 2.078 \times 10^{-144}$ . The author considered the gain in confidence which might attach to multiplying together the members of the ten remaining reciprocal pairs having the respective values of  $k = 1/96, \dots, = 1/24, = 1/12, \dots, = 1/3$  to be negligible. He felt justified in guaranteeing the accuracy of 136 overall figures for all of the 56 numbers in tables 1, 2 and 3, and in supposing that 138 figures may well be reliable if the last digits be rounded off conventionally. He also deemed it superfluous to present a full set of complex equivalents such as  $e^{-\pi/2} = i^i$ ,  $e^{-\pi/4} = i^{i/2} = (1 + i)^{i/2} / 2^{i/2}$ ,  $e^{-\pi/6} = i^{i/3} = (\sqrt{3} + i)^{i/2} / 2^i$ , etc.

It is now appropriate to compare the short table<sup>3</sup> published for the author in the year 1921 with the new table 1. In terms of unity in the 53rd decimal place the errors are  $+0.04, -0.6, -0.5, +0.5, -1.6, +0.6, -3.9, +0.6, -0.4$  and  $+2.4$ , respectively, for  $\cosh (\pi/6)$  or  $\sigma_1$ ,  $\sinh (\pi/6)$  or  $\sigma_2$ ,  $e^{+\pi/6}$ ,  $e^{-\pi/6}$ ,  $e^{+\pi/3}$ ,  $e^{-\pi/3}$ ,  $e^{+\pi/2}$ ,  $e^{-\pi/2}$ ,  $e^{+\pi}$  and  $e^{-\pi}$ . Hence, the chief comment on accuracy by the author in his earlier paper, to wit: "It is concluded that the calculated values of  $e^{\pi/6}$  and  $e^{-(\pi/6)}$  given above are certainly correct to 52 decimal places and they are probably in error by not more than two units in the fifty-third place." was too cautious since the estimated two units have just been shown to be a half-unit for each of these two constants.

With regard to the entries in tables 2 and 3 the only particular remark to be made is that the relation  $\cosh^2 x - \sinh^2 x = 1$  was not employed since it does not afford a significant check. Any pair of numbers  $c$  and  $s$  which are computed from  $c = (m + n)/2$  and  $s = (m - n)/2$  will automatically satisfy the condition  $c^2 - s^2 = 1$  when  $m$  and  $n$  are mutually reciprocal ( $mn = 1$ ) regardless of whether  $m$  and  $n$  are correct or false. The data recorded in tables 2 and 3 were finally tested, respectively, by comparing  $\cosh (k\pi) + \sinh (k\pi)$  with  $e^{+k\pi}$  and  $\cosh (k\pi) - \sinh (k\pi)$  with  $e^{-k\pi}$ .

TABLE 1

$e^{-\pi}$									
0.043213	91826	37722	49774	41773	71717	28011	27572	81098	10633
08298	07196	87401	05076	57570	17967	69813	99599	61901	08438
70168	06964	59766	20563	26519	83177	96586	643		
$e^{-\pi/2}$									
0.20787	95763	50761	90854	69556	19834	97877	00338	77841	63176
96080	75135	88305	54198	77285	48213	97886	00277	86542	60353
40521	77330	72350	21808	19061	97303	74663	987		
$e^{-\pi/3}$									
0.35091	98071	78410	96756	57867	15996	95305	83625	73153	62096
17406	52384	89783	45471	91387	90591	77985	90909	88323	39147
06666	33285	85352	68847	09190	14152	65579	259		

$$e^{-\pi/4}$$

0.45593	81277	65996	23676	59212	94728	02941	94166	04365	23785
18699	96290	97942	91596	45224	44006	65252	86099	31970	89010
89717	15729	22467	00469	21645	96570	72613	676		

$$e^{-\pi/6}$$

0.59238	48471	88388	98366	54163	32661	91948	74141	14145	73545
87885	86614	33131	34832	38837	31793	49841	44951	21797	99141
76059	55032	23110	94028	40466	25757	73779	619		

$$e^{-\pi/8}$$

0.67523	19066	55777	21703	20677	47365	67745	52428	58398	76998
90153	62810	86630	75497	42403	88968	02189	34997	39154	35601
82505	66947	52247	18507	06691	11544	94852	116		

$$e^{-\pi/12}$$

0.76966	54124	93239	80757	37687	07230	48101	92320	94381	45579
43945	37330	57085	91181	65585	87353	79624	51895	06972	17517
05126	94148	36376	35938	19214	09114	54172	251		

$$e^{-\pi/16}$$

0.82172	49580	33877	18208	07042	67874	56518	14786	26393	88136
50546	82104	78626	67328	29717	63362	32248	04748	72616	77689
30630	20435	02711	43458	19195	75361	79638	557		

$$e^{-\pi/24}$$

0.87730	57690	98345	66958	38146	46330	33330	36446	41138	05807
96653	77824	91012	19286	58909	33398	97527	78407	10063	25151
10456	22895	36087	95217	57535	38395	83850	623		

$$e^{-\pi/32}$$

0.90649	04621	85828	61749	19356	14428	78207	71753	10703	01884
27954	51723	37437	17268	52109	56926	31641	32136	87064	04019
48480	80078	74342	73205	24724	88735	18654	607		

$$e^{-\pi/48}$$

0.93664	60212	36595	90634	60522	74229	76655	38448	83531	30346
03214	97622	82015	35203	78452	73519	13683	08268	43321	87011
69933	53289	27014	10931	93187	77821	07073	897		

$$e^{-\pi/64}$$

0.95209	79267	83704	64850	57371	28524	78217	47368	50709	61530
20059	23646	99060	73544	68178	19136	92627	70109	16779	60607
15174	75247	57469	65910	64497	44635	78478	952		

$$e^{-\pi/96}$$

0.96780	47433	42682	68575	15312	11040	92577	36808	13730	06534
88551	75905	15613	56933	08794	76890	34445	46481	68610	49965
58229	25442	11221	67359	42414	34417	10376	428		

$$e^{-\pi/192}$$

0.98377	06761	95770	07076	45433	46905	86541	93981	56612	48425
61804	73315	01861	14284	86299	88069	84503	08483	02298	55201
10031	84251	03613	06973	17061	46654	75682	679		

$e + \pi/192$									
1.01649	70599	31678	93392	79156	91977	04841	01851	69753	48016
45221	51728	77947	03160	59876	08158	41919	12811	78737	71911
40026	17671	74146	56594	21738	12419	24174	12		
$e + \pi/96$									
1.03326	62728	49747	27440	79888	73714	29670	23989	95177	65525
96632	08760	80487	10875	83255	87611	11374	91682	69932	52813
67847	09374	25505	72658	50611	91924	40386	09		
$e + \pi/64$									
1.05031	21284	78332	07288	13100	54743	31723	52734	41557	23629
70275	41810	21062	57144	78851	52191	38322	78172	47476	78070
74928	68061	37557	46854	30037	05370	40045	69		
$e + \pi/48$									
1.06763	91906	08808	38146	14777	71484	30623	62679	69083	97978
68746	03773	54711	14283	14075	56843	00682	22767	51172	82414
77516	01271	92554	03224	88574	98282	14958	54		
$e + \pi/32$									
1.10315	55672	28684	33894	60412	81997	43095	22145	66262	92396
02550	30971	16357	39579	78763	16667	05051	27890	54487	28637
21238	67293	93445	02833	93112	75787	09558	62		
$e + \pi/24$									
1.13985	34413	23831	47486	81339	33638	76384	66648	24898	32557
63167	24018	88289	99447	14199	93899	39466	02696	23422	47749
77947	06700	11765	10426	61694	65093	26266	27		
$e + \pi/16$									
1.21695	22055	07640	29224	03772	32573	75356	27507	21240	12904
30191	15215	39645	38850	39433	17871	43394	16971	10496	70703
18256	14080	33875	12990	53549	49519	84234	55		
$e + \pi/12$									
1.29926	58676	97781	32296	99617	95129	29210	26875	68754	77964
06977	40006	55841	88751	53530	32172	97802	77089	46254	42062
48874	34933	84293	47909	62294	29885	28814	02		
$e + \pi/8$									
1.48097	26704	89909	97123	52415	92730	75004	77577	57711	23219
60912	61162	58951	82273	93344	38056	13382	19034	61143	69902
18669	03919	22545	01536	79608	24540	28835	45		
$e + \pi/6$									
1.68809	17949	64468	60061	68476	28096	78229	41196	81189	28718
23125	68405	60788	89433	93169	66280	20423	23490	95967	58606
00207	57323	50767	66184	69723	33214	55304	54		
$e + \pi/4$									
2.19328	00507	38015	45655	97696	59278	73822	34616	37641	99427
23348	58015	91865	70268	64189	28698	41265	22812	57816	94047
11677	59357	96761	56946	47041	60085	07626	05		

2.84965	39082	26361	49747	41273	19852	90499	39640	06102	78112
68817	43238	77848	81424	49158	84145	05473	44562	77101	70814
93266	95773	11240	85932	40160	96305	55410	98		

 $e^{+\pi/2}$ 

4.81047	73809	65351	65547	30356	66703	83312	63901	70874	66453
49400	20815	48924	25519	04891	58213	67487	04766	58388	33546
57282	22273	56991	29262	20334	57206	46343	99		

 $e^{+\pi}$ 

23.14069	26327	79269	00572	90863	67948	54738	02661	06242	60021
19934	45046	40952	43423	50690	45278	35169	71997	06754	92196
75952	70480	10877	73144	42804	44146	93835	8		

TABLE 2

 $\sinh (\pi/192)$ 

0.016363	19186	79544	31581	68617	25355	91495	39350	65704	97954
18583	92068	80429	44378	67881	00442	87080	21643	82195	83551
49971	67103	52667	48105	23383	28822	42457	185		

 $\sinh (\pi/96)$ 

0.032730	76475	35322	94328	22883	13366	85464	35909	07237	94955
40401	64278	24367	69711	22305	53603	84647	26005	31610	14240
48089	19660	71420	26495	40987	87536	50048	297		

 $\sinh (\pi/64)$ 

0.049107	10084	73137	12187	78646	31092	67530	26829	54238	10497
51080	90816	10009	18000	53366	65272	28475	40316	53485	87317
98769	64069	00439	04718	27698	03673	07833	702		

 $\sinh (\pi/48)$ 

0.065496	58468	61062	37557	71274	86272	69841	21154	27763	38163
27655	30753	63478	95396	78114	16619	34995	72495	39254	77015
37912	39913	27699	61464	76936	02305	39423	205		

 $\sinh (\pi/32)$ 

0.098332	55252	14278	60727	05283	37843	24437	51962	77799	52558
72978	96238	94601	11556	33267	98703	67049	78768	37116	23088
63789	36075	95511	48143	41939	35259	54520	049		

 $\sinh (\pi/24)$ 

0.13127	38361	12742	90264	21596	43654	21527	15100	91880	13374
83256	73096	98638	90080	27645	30250	20969	12144	56679	61299
33745	41902	37838	57604	52079	63348	71207	823		

 $\sinh (\pi/16)$ 

0.19761	36237	36881	55477	98364	82349	59419	06360	47423	12383
89822	16555	80509	35761	04857	77254	55573	06111	18939	96506
93812	96822	65581	84766	17176	87079	02297	995		

 $\sinh (\pi/12)$ 

0.26480	02276	02270	75769	80965	43949	40554	17277	37186	66192
31516	01337	99377	98784	93972	22409	59089	12596	89641	12272
71873	70392	73958	55985	71540	10385	37320	884		

sinh ( $\pi/8$ )									
0.40287	03819	17066	37710	15869	22682	53629	62574	49656	23110
35379	49175	86160	53388	25470	24544	05596	42018	60994	67150
18081	68485	85148	91514	56458	56497	66991	665		
sinh ( $\pi/6$ )									
0.54785	34738	88039	80847	57156	47717	43140	33527	83521	77586
17619	90895	63828	77300	77166	17243	35290	89264	87084	79732
12074	01145	63828	36078	14628	53728	40762	462		
sinh ( $\pi/4$ )									
0.86867	09614	86009	60989	69241	82275	35440	20225	16638	37821
02324	30862	46961	39336	09482	39843	38006	18356	62923	02518
10980	21814	34147	28238	62697	81757	17506	189		
sinh ( $\pi/3$ )									
1.24936	70505	23975	26495	41953	01927	97566	78007	16474	58008
25705	45426	94032	67976	28885	46776	63743	76826	44389	15833
93300	31243	62944	08542	65485	41076	44915	86		
sinh ( $\pi/2$ )									
2.30129	89023	07294	87346	30400	23434	42717	81781	46516	51638
26659	72839	80309	35660	13803	04999	84800	52244	35922	86596
58380	22471	42320	53727	00636	29951	35840	00		
sinh ( $\pi$ )									
11.54873	93572	57748	37797	73343	15388	40968	44951	89066	39478
94552	32163	36106	16457	92466	71740	79094	16018	55282	40676
44467	94891	82450	55544	05076	22914	57088	6		

TABLE 3

cosh ( $\pi/192$ )									
1.00013	38680	63724	50234	62295	19441	45691	47916	63182	98221
03363	12521	89904	08722	73087	98114	13211	10647	40518	13556
25029	00961	38879	81783	69399	79536	99928	40		
cosh ( $\pi/96$ )									
1.00053	55080	96214	98007	97600	42377	61123	80399	04458	86030
42591	92332	98050	33904	21025	32250	72910	19082	16771	51389
63038	17408	18363	70008	96513	13170	75381	26		
cosh ( $\pi/64$ )									
1.00120	50276	31018	36069	35235	91634	04970	50051	46133	42579
95167	32728	60061	65344	73514	85664	15475	24140	82128	19338
95051	71654	47513	56382	47267	25003	09262	32		
cosh ( $\pi/48$ )									
1.00214	26059	22702	14390	37650	22857	03639	50564	26307	64162
35980	50698	18363	24743	46264	15181	07182	65517	97247	34718
23724	77280	59784	07078	40881	38051	61016	22		
cosh ( $\pi/32$ )									
1.00482	30147	07256	47821	89884	48213	10651	46949	38482	97140
15252	41347	26897	28424	15436	86796	68346	60013	70775	66328
34859	73686	33893	88019	58918	82261	14106	61		

cosh ( $\pi/24$ )									
1.00857	96052	11088	57222	59742	89984	54857	51547	33018	19182
79910	50921	89651	09366	86554	63649	18496	90551	66742	86450
44201	64797	73926	52822	09615	01744	55058	45		
cosh ( $\pi/16$ )									
1.01933	85817	70758	73746	05407	50224	15937	21146	73817	00520
40368	98660	09136	03089	34575	40616	87821	10859	91556	74196
24443	17257	68293	28224	36372	62440	81936	55		
cosh ( $\pi/12$ )									
1.03446	56400	95510	56527	18652	51179	88656	09598	31568	11771
75461	38668	56463	89966	59558	09763	38713	64492	56613	29789
77000	64541	10334	91923	90754	19499	91493	13		
cosh ( $\pi/8$ )									
1.07810	22885	72843	59413	36546	70048	21375	15003	08055	00109
25533	11986	72791	28885	67874	13512	07785	77016	00149	02752
00587	35433	37396	10022	23149	68042	61843	78		
cosh ( $\pi/6$ )									
1.14023	83210	76428	79214	11319	80379	35089	07668	97667	51132
05505	77509	96960	12133	16003	49036	85132	34216	08882	78873
88133	56177	86939	30106	55094	79486	14542	08		
cosh ( $\pi/4$ )									
1.32460	90892	52005	84666	28454	77003	38382	14391	21003	61606
21024	27153	44904	30932	54706	83850	03259	04455	94893	91529
00697	37543	56614	28707	84343	78327	90119	86		
cosh ( $\pi/3$ )									
1.60028	68577	02386	23251	99320	17924	92872	61632	89628	20104
43111	97811	83816	13448	20273	37368	41729	67736	32712	54980
99966	64529	48296	77389	74675	55229	10495	12		
cosh ( $\pi/2$ )									
2.50917	84786	58056	78200	99956	43269	40594	82120	24358	14815
22740	47975	68614	89858	91088	53213	82686	52522	22465	46949
98901	99802	14670	75535	19698	27255	10503	99		
cosh ( $\pi$ )									
11.59195	32755	21520	62775	17520	52500	13769	57709	17176	20542
25382	12883	04846	26965	58223	73537	56075	55978	51472	51520
31484	75588	28427	17600	37728	21232	36747	2(5)		

<sup>1</sup> Fletcher, A., Miller, J. C. P., and Rosenhead, L., *An Index of Mathematical Tables*, London (1946).

<sup>2</sup> Uhler, H. S., *Amer. Math. Monthly*, 28, 114 (1921).

<sup>3</sup> *Ibid.*, 115.



## A REVERSE MUTATION TO A "REMOTE" ALLELE IN THE HOUSE MOUSE

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The gene  $A^w$  producing the white bellied agouti coat pattern in mice is one of a series of multiple alleles. Epistatic to it is the gene  $A^y$  for yellow coat color and hypostatic in descending order are:  $A$  = gray bellied agouti,  $a'$  = black and tan, and  $a$  = non-agouti.

There have been a number of recorded cases of mutations from other alleles of the agouti series to  $A^w$  (Little, 1916<sup>1</sup>; Keeler, 1931<sup>2</sup>). In each case the mutation was or could have been a "single-step" reverse mutation from  $A$ .

The fact that the genotype  $Aa'$  has synthetically a coat color practically identical with  $A^wA^w$  mice has led Pincus (1929)<sup>3</sup> and Keeler (*loc. cit.*) to raise the question as to whether there might be a dominant gene for white or light belly closely linked with the agouti locus. No crossovers have, however, been recorded.

As Gruneberg (1943, p. 30)<sup>4</sup> points out, a mutation from  $A^w$  to  $a$  or one in the reverse direction would provide strong evidence in favor of the hypothesis of multiple alleles and against that of linkage.

The occurrence of three  $A^wa$  individuals in different litters from a single mating of the closely inbred dilute brown ( $dba$ ) strain of mice which has previously produced only  $aa$  animals<sup>5</sup> since 1909 has been observed and is here recorded. The three individuals occurred among the progeny of ♀ 1 × ♂ 10, there being 18 normal  $aa$  young.

The three mutant young differed in coat color from their  $dba$  parents only in the change from  $a$  to  $A^w$ . One of the two young is still too young to breed. One died in infancy. The other ♀ 2 has been crossed with non-agouti  $aa$  individuals and has produced 6 white-bellied agouti  $A^wa$  individuals and 5 non-agouti  $aa$  thus showing that the genetic formula of the mutant ♀ 2 was  $A^wabbdd$  as would be expected if the genetic change occurred in only one of the  $dba$  parents.

The frequency of the appearance of the mutant mice in the original mating suggests that one gonad of the mutating parent is a mosaic in respect to the formation of  $A^w$  and  $a$  gametes.

An effort has been made to determine which parent is the animal thus affected. Since the female produced three litters by a dilute brown male in order to provide the primary data on which the occurrence of the mutation is based there has not been time as yet to make a significant quantitative study of her progeny by unrelated males.

Breeding tests of the father of the mutants have, however, been made. When crossed with unrelated non-agouti females ( $aa$ ) he has given a total of 43 young, all non-agouti. It, therefore, appears that he alone is not responsible for the appearance of the mutants. If one parent is responsible it is apparently the female.

There remains a theoretical possibility to be tested by further breeding. The ratio of non-mutant ( $aa$ ) to mutant ( $A^w a$ ) mice is 18 to 3. This approximates a theoretical 3:1 ratio of 15:5. If both parents carried a hypothetical gene ( $m$ ) which when contributed by both to the embryo caused a mutation from  $a$  to  $A^w$  in one of the pair of chromosomes in which the agouti locus is situated and if this mutation occurred in the  $mm$  embryos at or soon after fertilization a high incidence of mutants comparable to that observed would be expected.

The  $m$  gene would be carried by two-thirds of the  $aa$  mice produced from the mating of the original parents and in 50% of the  $aa$  mice produced from any outcross of either parent. Inbreeding such  $aa$  mice should recreate combinations which were  $mm$  and which, therefore, mutated. It may be admitted that this explanation is somewhat improbable but the high relative incidence of the mutation among the original sibship is very unusual when compared with previous records of dominant mutation incidence in rodents and it seems desirable to record any possible explanation which might account for it.

The present case is interesting because of the following facts:

(1) It represents a reverse mutation which has apparently "skipped" two alleles ( $a'$  and  $A$ ) epistatic to  $a$  in order to reach the  $A^w$  allele. In such a multiple allelomorphic series it is evident that the configuration of the gene  $a$  can become that of its allele  $A^w$  without evidence of its having passed through the two genetically "interstatic" alleles,  $a'$  and  $A$ .

(2) Its frequency of appearance is sufficient to suggest a very early division of the gonad of the mutating parent into " $A^w a$ " and " $aa$ " bearing cells.

(3) It makes the truly multiple allelomorphic series of  $A^v$ ,  $A^w$ ,  $A$ ,  $a'$  and  $a$  appear much more probable than the hypothesis of close linkage of a gene for "light belly" with the "agouti" locus.

<sup>1</sup> Little, C. C., *Am. Nat.*, 50, 335-349 (1916).

<sup>2</sup> Keeler, C. E., *Proc. Nat. Acad. Sci.*, 17, 497-499 (1931).

<sup>3</sup> Pincus, G., *Proc. Nat. Acad. Sci.*, 15, 85-88 (1929).

<sup>4</sup> Gruneberg, H., *The Genetics of the Mouse*, Cambridge Univ. Press, pp. xii + 412 (1943).

<sup>5</sup> Other color mutations in the *dba* strain have been recorded as follows: (1) from  $P$  to  $p$ —dark eye to pink eye, Little, 1916; (2) from self coat  $Bt$  to white belted  $bt$ , Murray, 1936; (3) from normal pigmentation to a new type of dilution "misty," Woolley, 1945. Two other "dilute" mutants—one of them possibly identical with the "leaden" type of pigment production—are now being investigated.

## CHROMOSOMES OF THE MINK\*

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Chromosomes of only one of the Mustelidae have been studied in so far as the writers are aware. Koller,<sup>2</sup> working with the testes of both oestrous and anoestrous individuals, found the diploid chromosome number of the ferret (*Putorius furo*, L.) to be 34. He observed an unequal pair which he considered the sex chromosomes, the Y chromosome being "3-4 times smaller" than the X chromosome. He stated further that the sex chromosomes were "precocious in their behavior during meiotic prophase," and that the "XY bivalent lies off the metaphase plate."

The present report is concerned with the number and general morphology of the chromosomes of the standard dark color phase of ranch-bred mink (*Mustela vison*, Peale and Beauvois). Testes from six animals were removed in March and April (three animals in 1942, one in 1943 and two in 1946), and the seminiferous tubules were immediately teased out and placed in Carnoy's alcohol-acetic acid-chloroform solution (7:2:1). Since speedy fixation is necessary for the immediate arrest of division figures, the operation was completed in less than five minutes for each pair of testes. The tubules were allowed to remain in the fixative from 30 minutes to one hour, then stored in 80 per cent alcohol. Temporary mounts prepared by the aceto-carminic smear method were made from this material. Polar views of diploid equatorial plates show 28 to be the chromosome number of the mink (Fig. 1A). The chromosomes vary in length, the shortest being about one-third that of the longest. They are longitudinally split with median or subterminal spindle-attachment regions.

Mitotic as well as meiotic divisions are relatively abundant in the seminiferous tubules of the testes of mink during the breeding season. Homologous pairs of chromosomes are easily identified in several instances. In order to study their comparative morphology, each of the chromosomes has been arranged with what appears to be its counterpart using total length, position of the spindle-attachment region and relative lengths of the two arms as a guide (Fig. 1B). Thirteen pairs match as if they are homologous chromosomes. The two chromosomes of the fourteenth

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FIGURE 1A. Mitotic equatorial plate showing the diploid chromosome number of 28.

FIGURE 1B. Chromosomes from 1A arranged as homologous pairs.

FIGURE 2. Late heterotypic prophase, showing the chromosome with the median attached satellite (*s*), and the nucleolus (*n*).

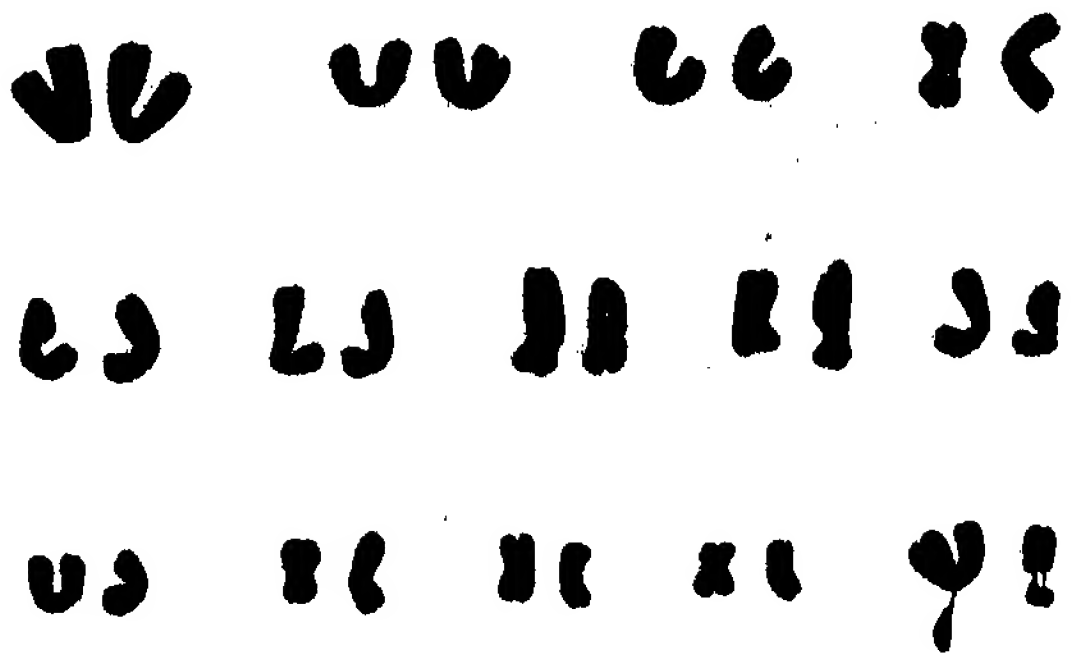
FIGURE 3. Polar view of a homeotypic equatorial plate.

FIGURE 4. Side view of heterotypic chromosomes approaching the equatorial plate. Two chromosomes show chiasmata (*c*). Note astral formations.

(Remainder of legend at bottom of p. 45)



1 A



1 B



2



3



5



4



6

FIGURE 5. Heterotypic chromosomes at anaphase.  
FIGURE 6. Mitotic chromosomes at anaphase.  
All figures  $\times 2100$ .

pair are notably different. One has a subterminal spindle-attachment region and a terminal satellite. It appears to be much like the satellited pair found in the fox and in many plants.<sup>5</sup> The other has a median spindle-attachment region and the satellite is connected at the same region as the spindle fibre (Fig. 1B). The chromosome itself is almost twice the length of its satellited mate. The similarity between these two chromosomes is striking if one considers the possibility of the smaller having arisen from the larger by the loss of an arm. Chromosomes with median attached satellites have been found in *Tradescantia* by Darlington<sup>1</sup> and in *Allium* by Levan,<sup>3</sup> and with laterally attached satellites in *Agrostis* by Tinney.<sup>4</sup> As a general rule, satellited chromosomes of both plants and animals are found associated with the nucleolus. This does not seem to hold true in the mink, at least not for the median satellited chromosome (Fig. 2). Neither Levan nor Tinney found any relationship between the chromosomes with median or laterally attached satellites and the nucleoli. Since the male is known to be the heterogametic sex in many mammals, the satellited pair of chromosomes may represent the sex chromosomes of the mink. Thus far, no satisfactory chromosome configuration from female tissues has been obtained.

The haploid number of chromosomes is more difficult to determine because the chromosomes are shorter, thicker and more closely associated on the meiotic equatorial plates than in the case of a mitotic metaphase. However, the 14 chromosomes on the equatorial plate of the second meiotic division confirms the diploid count herein reported (Fig. 3).

Spindle fibres and the resulting astral formations are clearly revealed by the aceto-carmin staining method (Fig. 4). The chromosomes in Figure 4 are approaching the equatorial plate for the first meiotic division, and chiasmata are to be seen in two of the chromosome pairs (Fig. 4C). No evidence of either precocious or lagging chromosomes was found in meiotic or mitotic anaphases (compare Figs. 5 and 6).

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<sup>1</sup> Darlington, C. D., *Jour. Genetics*, 21, 207-286 (1929).

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<sup>4</sup> Tinney, F. W., *Bot. Gaz.*, 97, 822-833 (1936).

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# PROCEEDINGS OF THE NATIONAL ACADEMY OF SCIENCES

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## CURVATURE THEOREMS ON POLAR CURVES\*

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1. The principle of duality in projective geometry is based on the theory of poles and polars with respect to a conic. For a conic, a point has only one kind of polar, the first polar, or polar straight line. However, for a general algebraic curve of higher degree  $n$ , a point has not only the first polar of degree  $n - 1$ , but also the second polar of degree  $n - 2$ , ..., the  $r$ th polar of degree  $n - r$ , ..., the  $(n - 1)$  polar of degree 1. This last polar is a straight line. The general polar theory<sup>1</sup> goes back to Newton, and was developed by Bobillier, Cayley, Salmon, Clebsch, Aronhold, Clifford and Mayer.

For a given curve  $C_n$  of degree  $n$ , the first polar of any point  $O$  of the plane is a curve  $C_{n-1}$  of degree  $n - 1$ . If  $O$  is a point of  $C_n$ , it is well known that the polar curve  $C_{n-1}$  passes through  $O$  and touches the given curve  $C_n$  at  $O$ . However, *the two curves do not have the same curvature*. We find that the ratio  $\rho_1$  of the curvature of  $C_{n-1}$  to that of  $C_n$  is  $\rho_1 = (n - 2)/(n - 1)$ . For example, if the given curve is a cubic curve  $C_3$ , the first polar is a conic  $C_2$ , and at an ordinary point  $O$  of  $C_3$ , the ratio of the curvatures is  $1/2$ . These simple theorems appear to be new.

We generalize this for all the higher polars. All the higher polars go through the given point  $O$  of the curve  $C_n$  and touch  $C_n$  at  $O$ . In particular, the last polar coincides with the tangent line at  $O$ . The curvatures at  $O$  all have different values (the last one being obviously zero), and we determine all these curvatures

In the above discussion, we assumed that  $O$  is an ordinary point of the algebraic curve  $C_n$ . We conclude by discussing also the theory at a singular point  $O$  of  $C_n$ ; for example, inflections and cusps.

Although our new theorems are stated in metric terms, they are essentially theorems of differential projective geometry. This is a consequence of the theorem of Mehmke-Segre which states that if any two differ-



entiable curves are tangent at a given point 0, then the ratio  $\rho$  of their departures from the common tangent line is a projective invariant.

2. An algebraic curve  $C_n$  of degree  $n$  is defined in cartesian coördinates  $(x, y)$  by the equation

$$C_n: \phi(x, y) = \sum_{k=0}^n P_k(x, y) = 0, \quad (1)$$

where  $P_k(x, y)$  is a homogeneous polynomial in  $(x, y)$  of degree  $k$ , and  $P_n(x, y)$  is not identically zero. It is convenient to use non-homogeneous cartesian coordinates  $(x, y)$  since we are dealing with the metric character of the polar curves.

Upon taking the given point 0 at the origin, we find that the first polar  $C_{n-1}$  of degree  $n - 1$  of 0 with respect to  $C_n$ , is<sup>2</sup>

$$C_{n-1}: \sum_{k=0}^{n-1} (n - k) P_k(x, y) = 0. \quad (2)$$

The  $r$ th polar  $C_{n-r}$  of degree  $n - r$  of the point 0 with respect to  $C_n$  may be defined by induction. It is the first polar of the same point 0 with respect to the  $(r - 1)$  polar  $C_{n-r+1}$  of degree  $n - r + 1$ . Thus the equation of the  $r$ th polar of the origin 0 is

$$C_{n-r}: \sum_{k=0}^{n-r} (n - k)(n - k - 1) \dots (n - k - r + 1) P_k(x, y) = 0. \quad (3)$$

The  $(n - 1)$  polar  $C_1$  of the origin 0 is the polar line

$$C_1: nP_0 + P_1(x, y) = 0. \quad (4)$$

3. Let the origin 0 be an ordinary point of the algebraic curve  $C_n$  so that  $P_0 = 0$  and  $P_1(x, y)$  is not identically zero. The tangent line of  $C_n$  at 0 is given by the equation  $P_1(x, y) = 0$ , and hence is identical with the polar line (4). By equations (2) and (3), it is seen that all the polar curves of 0 with respect to  $C_n$  pass through 0 and touch  $C_n$  at 0.

**THEOREM 1.** *The ratio  $\rho_1$  of the curvature of the first polar  $C_{n-1}$  to the curvature of  $C_n$ , constructed at an ordinary point 0 of  $C_n$ , is*

$$\rho_1 = \frac{n - 2}{n - 1}. \quad (5)$$

This can be deduced from equations (1) and (2). By considering the equations (1) and (3), we obtain the following generalization. This can be also obtained by iteration.

**THEOREM 2.** *The ratio  $\rho_r$  of the curvature of the  $r$ th polar curve  $C_{n-r}$  to the curvature of the algebraic curve  $C_n$ , evaluated at an ordinary point 0 of  $C_n$  is*

$$\rho_r = \frac{n - r - 1}{n - 1}. \quad (6)$$

In particular, consider a quartic curve  $C_4$ . At an ordinary point  $O$  of  $C_4$ , construct the polar cubic  $C_3$ , the polar conic  $C_2$ , and the polar line  $C_1$ . These all touch  $C_4$  at  $O$  and the corresponding ratios of the curvatures are  $2/3$ ,  $1/3$ ,  $0$ .

4. Before continuing with our work, it is necessary to consider some definitions. At a point  $P$  of a curve  $C$ , construct the tangent line  $t$ . From a point  $Q$  on the curve  $C$  near  $P$ , draw a line perpendicular to  $t$  and intersecting  $t$  in the point  $R$ . The *order of contact*  $\gamma$  of the curve  $C$  with its tangent line  $t$  at  $P$  is the positive number  $\gamma$  for which  $\lim_{R \rightarrow P} \overline{RQ}/(\overline{PR})^{\gamma+1}$ , is a finite non-zero number. It is remarked that  $\gamma$  is any positive real number.

Let two curves  $C_1$  and  $C_2$  be tangent at a point  $P$ . Through a point  $R$  on the common tangent line  $t$  near  $P$ , erect a perpendicular to  $t$ , which intersects the two curves  $C_1$  and  $C_2$  in the points  $Q_1$  and  $Q_2$ , both of which are near  $P$ . The *ratio  $\rho$  of the departure of  $C_2$  to that of  $C_1$  from the common tangent line  $t$*  is defined by the expression:  $\rho = \lim_{R \rightarrow P} \overline{RQ_2}/\overline{RQ_1}$ . If  $\kappa^{(r)}$  denotes the  $r$ th derivative of the curvature  $\kappa$  of a curve  $C$  with respect to its arc length, and if  $\gamma$ , the order of contact of  $C_1$  or  $C_2$  with the common tangent line  $t$  at  $P$ , is a positive integer, then it can be shown that  $\rho = \kappa_2^{(\gamma-1)}/\kappa_1^{(\gamma-1)}$ . According to the theorem of Mehnke-Segre, this ratio  $\rho$  is a projective invariant.

5. By the equations (1), (2) and (3), it is found that if  $O$  is an ordinary point of the algebraic curve  $C_n$ , and if the order of contact of  $C_n$  with its tangent line at  $O$  is  $\gamma$ , then all the polar curves of  $O$  with respect to  $C_n$  are tangent to  $C_n$  at  $O$ , and the order of contact of each of these polars with the common tangent line is also  $\gamma$ .

**THEOREM 3.** *The ratio  $\rho_r$  of the departure of the  $r$ th polar curve  $C_{n-r}$  to the departure of  $C_n$ , constructed at a point  $O$  of  $C_n$  in the case where the order of contact with the common tangent line at  $O$  is  $\gamma$  which may be any positive integer, is*

$$\rho_r = \frac{(n - \gamma - 1)(n - \gamma - 2) \dots (n - \gamma - r)}{(n - 1)(n - 2) \dots (n - r)}. \quad (7)$$

*In particular, the corresponding ratios  $\rho_1$ ,  $\rho_2$ ,  $\rho_3$  for the first, second and third polars are*

$$\begin{aligned} \rho_1 &= \frac{n - \gamma - 1}{n - 1}, & \rho_2 &= \frac{(n - \gamma - 1)(n - \gamma - 2)}{(n - 1)(n - 2)}, \\ \rho_3 &= \frac{(n - \gamma - 1)(n - \gamma - 2)(n - \gamma - 3)}{(n - 1)(n - 2)(n - 3)}. \end{aligned} \quad (8)$$

6. Now we consider the case where the origin 0 is a cusp of lowest order of the algebraic curve  $C_n$ . Then in equation (1), we must have that  $P_0 = 0$ ,  $P_1(x, y)$  is identically zero, and  $P_2(x, y)$  is a constant non-zero multiple of the square of a linear homogeneous form in  $(x, y)$ . From equations (2) and (3), it is deduced that all the polars ( $r = 1, 2, \dots, n - 2$ ) have a cusp of the same qualitative nature at 0.

**THEOREM 4.** *The ratio  $\rho_r$  of the departure of the  $r$ th polar  $C_{n-r}$  to the departure of  $C_n$ , constructed at a simple cusp 0 of  $C_n$  in the case where the order of contact is  $1/2$ , is*

$$\rho_r = \left( \frac{n - r - 2}{n - 2} \right)^{1/2}. \quad (9)$$

*In particular, the corresponding ratios  $\rho_1, \rho_2, \rho_3$  for the first, second and third polars are*

$$\rho_1 = \left( \frac{n - 3}{n - 2} \right)^{1/2}, \quad \rho_2 = \left( \frac{n - 4}{n - 2} \right)^{1/2}, \quad \rho_3 = \left( \frac{n - 5}{n - 2} \right)^{1/2}. \quad (10)$$

7. In this section, we study the ratio  $\rho_r$  constructed at a simple cusp 0 of the algebraic curve  $C_n$  in the case where the order of contact with the common tangent line is greater than  $1/2$ . We have proved the following result.

**THEOREM 5.** *Let 0 be a simple cusp of the algebraic curve  $C_n$  so that the order of contact  $\gamma$  of  $C_n$  with the tangent line at 0 is an integral multiple of  $1/2$ . The  $r$ th polar  $C_{n-r}$  of 0 with respect to  $C_n$  also has a simple cusp at 0 with the same tangent line and the same order of contact  $\gamma$  at 0. If  $\gamma = (2q - 1)/2$ , the ratio  $\rho_r$  of the departure of  $C_{n-r}$  to the departure of  $C_n$  from the common tangent line at 0 is*

$$\rho_r = \left[ \frac{(n - 2q - 1)(n - 2q - 2) \dots (n - 2q - r)}{(n - 2)(n - 3) \dots (n - 1 - r)} \right]^{1/2}. \quad (11)$$

*In particular, the corresponding ratios  $\rho_1, \rho_2, \rho_3$  for the first, second and third polars are*

$$\begin{aligned} \rho_1 &= \left( \frac{n - 2q - 1}{n - 2} \right)^{1/2}, & \rho_2 &= \left[ \frac{(n - 2q - 1)(n - 2q - 2)}{(n - 2)(n - 3)} \right]^{1/2}, \\ \rho_3 &= \left[ \frac{(n - 2q - 1)(n - 2q - 2)(n - 2q - 3)}{(n - 2)(n - 3)(n - 4)} \right]^{1/2} \end{aligned} \quad (12)$$

On the other hand, if  $\gamma$  is an integer, then  $\rho_r$  depends on the coefficients of the polynomial equation defining the algebraic curve  $C_n$ .

It is remarked that if 0 is a simple cusp where the order of contact is a positive integer  $\gamma$ , we meet for the first time a case where the ratio  $\rho_r$  is

not an arithmetic invariant but actually is a differential invariant, that is,  $\rho_r$  depends on the coefficients of the original algebraic curve  $C_n$ .

8. In our later work, we shall study this ratio  $\rho_r$  for a singular point 0 of higher order of the algebraic curve  $C_n$ . We shall generalize the results of this article to algebraic surfaces and to higher manifolds in later papers.

Our theorems since they deal with curvature appear to belong to differential geometry, that is, geometry in the small. But actually the notion of polar curve involves the total algebraic curve and therefore our theorems belong to geometry in the large, that is, to global geometry.

\* Presented to the American Mathematical Society, Feb. 1947.

<sup>1</sup> Salmon, *Treatise on Higher Plane Curves* (1852).

<sup>2</sup> Kasner, "Determination of the Algebraic Curves Whose Polar Curves Are Parabolas," *Amer. Jour. Math.*, 26, 164-168 (1903).

## WEAK COMPACTNESS IN BANACH SPACES I

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1. In recent years the "weak" topology of Banach spaces has aroused interest, principally because a notion of weak compactness is vital to the theory of infinite dimensional linear spaces. Advances in the compactness theory, however, have been retarded by apparent dichotomy in nomenclature—"compactness" versus "bicomcompactness." From the standpoint of existing applications the basic tool is the weakly convergent subsequence or "compactness"; from the standpoint of topological structure the concept of "bicomcompactness" is fundamental. We prove that for weakly closed subsets of *any* Banach space these two seemingly disparate notions are one.

2. Let  $E$  be any Banach space with elements  $x$  and unit sphere  $S$ ,  $E^*$  the conjugate space with elements  $f$  and unit sphere  $S^*$ , and  $E^{**}$  the second conjugate space with elements  $X$ . Denote by  $E_\tau$  the linear topological space (weak topology of  $E$ ) formed by the elements of  $E$  under the neighborhood system  $U(x_0) \equiv [x \mid |f_i(x - x_0)| < \epsilon, i = 1, \dots, n]$ , where the  $f_i$  are arbitrary elements of  $E^*$ . A like topology can be introduced in  $E^*$  by taking elements from  $E^{**}$ , but a more functional topological space  ${}_\tau E^*$  (weak\* topology of  $E^*$ ) arises from the generic neighborhoods  $U(f_0) \equiv [f \mid |f(x_i) - f_0(x_i)| < \epsilon, i = 1, \dots, n]$ , where the  $x_i$  are arbitrary elements of  $E$ . A space  ${}_\tau E^{**} = {}_\tau(E^*)^*$  (weak\* topology of  $E^{**}$ ) is similarly generated; and the mapping  $J(x) = X$  defined by  $X(f) = f(x)$  imbeds  $E$

isometrically in  $E^{**}$  and  $E_T$  in  ${}_TE^{**}$ . We shall regard this imbedding as implicitly performed whenever convenient. Our key lemma then takes the form of a necessary and sufficient condition that an element  $X$  of  $E^{**}$  lie in  $E$ .

The principal justification for the weak\* topology is contained in the Tychonoff-Alaoglu<sup>1</sup> theorem:  $S^*$  is a bicomact subset of  ${}_TE^*$ . A further result we need is the equivalence<sup>2</sup> (for linear sets in  $E^*$ ) of the properties of regular closure,<sup>3</sup> weak\* closure, and, most important of all, "bounded" weak\* closure—variously referred to as transfinite closure or weak\* completeness. The contact with our work is the observation, implicit in the Banach<sup>4</sup> development, that an  $X$  of  $E^{**}$  lies in  $E$  if and only if the set  $Q_X \equiv \{f \mid X(f) = 0\}$  is regularly closed in  $E^*$ .

We call a set  $M$  in  $E_T$  compact (weakly compact in  $E$ ) if every infinite subset of  $M$  possesses at least one limit point  $x$  in  $E_T$ <sup>5</sup>; we call  $M$  sequentially compact if every infinite sequence in  $M$  contains a subsequence converging to a limit in  $E_T$ . Compactness implies the formally stronger property of sequential compactness<sup>6</sup> and the boundedness of  $M$  in the norm.<sup>7</sup>

LEMMA: *A necessary and sufficient condition that an element  $X$  of  $E^{**}$  lie in  $E$  is that there exist some weakly compact set  $M$  in  $E$  with the following property: Given arbitrary  $(f_1, \dots, f_n)$  in  $E^*$ , there is an  $x$  in  $M$  such that  $X(f_i) = f_i(x)$  ( $i = 1, \dots, n$ ).*

*Proof:* The necessity of the condition is trivial and, by virtue of the preceding remarks, establishing the sufficiency reduces to showing that if  $g$  in  $E^*$  is a weak\* limit point of  $Q_X \cdot S^*$ , then  $X(g) = 0$ .

Let  $\epsilon > 0$  be arbitrary. The hypothesis implies the existence of an  $x_0$  in  $M$  such that  $g(x_0) = X(g)$ . But then there exists an  $f_1$  in  $Q_X \cdot S^*$  such that  $|X(g) - f_1(x_0)| = |g(x_0) - f_1(x_0)| < \epsilon/2$ . By alternate appeal to the condition on  $M$  and the definition of weak\* limit point we thus construct a sequence  $(x_n)$  in  $M$  and a dual sequence  $(f_m)$  in  $Q_X \cdot S^*$  with the properties:

- (A)  $\|f_m\| \leq 1$  ( $m = 1, 2, \dots$ )
- (B)  $f_m(x_n) = 0$  ( $m = 1, \dots, n$ )
- (C)  $g(x_n) = X(g)$  ( $n = 0, 1, \dots$ )
- (D)  $|X(g) - f_m(x_n)| < \epsilon/2$  ( $n = 0, 1, \dots, m - 1$ )

The (sequential) weak compactness of  $M$  implies the existence of an  $x$  in  $E$  and a subsequence  $(x_{n_i})$  of  $(x_n)$  such that  $f(x) = \lim f(x_{n_i})$  for every  $f$  in  $E^*$ , whence  $f_m(x) = 0$  for all  $m$ . A corollary to the fundamental theorem of Mazur<sup>8</sup>—on the equivalence of weak and strong closure for

convex sets in  $E$ —provides an element  $z$  in  $E$  of the form  $z = \sum_{n=0}^N a_n x_n$  ( $a_n \geq 0$ ,  $\sum_{n=0}^N a_n = 1$ ), such that  $\|z - x\| < \epsilon/2$ . Now set  $m = N + 1$  in

(D). Multiplication of the  $N + 1$  inequalities by the proper  $a_n$ , addition, and the triangle inequality yield  $|X(g) - f_{N+1}(z)| < \epsilon/2$ . But then

$$|X(g)| = |X(g) - f_{N+1}(z) + f_{N+1}(z) - f_{N+1}(x)| < \epsilon.$$

Hence  $X(g) = 0$ , and the proof of the lemma is complete.

If we consider a compact (and therefore strongly bounded) set  $M$  closed in  $E_T$ , we find that the lemma implies its closure<sup>10</sup> in  ${}_\tau E^{**}$ . The Tychonoff-Alaoglu result then yields the non-trivial half of the

**THEOREM:** *A set  $M$  in  $E_T$  is bicomact if and only if it is compact and closed.*

An automatic consequence of the lemma, by way of the Helly theorem,<sup>9</sup> is the

**COROLLARY:**  *$E$  is reflexive— $J(E) = E^{**}$ —if and only if  $S$  is weakly compact.*

3. Our main result indicates that a number of special devices involving weak compactness can be replaced by a single bicomactness argument. For example, the mean ergodic theorem<sup>11</sup> for Abelian semi-groups of transformations reduces immediately to the  $r$ -parameter case, of which an integration-free treatment is possible. But deeper consequences in the ergodic theory, as well as extensions to topological groups and rings, are accessible. We hope to discuss these and other applications more fully in a later note.

<sup>1</sup> Tychonoff, A., "Über die topologische Erweiterung von Räumen," *Math. Annalen*, **102**, 544-561 (1930). Alaoglu, L., "Weak Topologies of Normed Linear Spaces," *Ann. Math.*, **41**, 252-267 (1940).

<sup>2</sup> See Alaoglu, *loc. cit.*, p. 256.

<sup>3</sup> Banach, S., "Théorie des Operations Linéaires," 1932, Chapitre VIII.

<sup>4</sup> Banach, *loc. cit.*, pp. 131-132.

<sup>5</sup> The closed convex extension of a compact set in  $E_T$  is again compact. See Phillips, R. S., "On Weakly Compact Subsets of a Banach Space," *Amer. J. Math.*, **65**, 108-136 (1943).

<sup>6</sup> Smulian, V., "Über lineare topologische Räume," *Mat. Sbornik N.S.*, **7** (49), 425-448 (1941). See also "Sur les ensembles régulièrement fermes and faiblement compact dans l'espace du type (B)," *C. R. (Doklady) Acad. Sci. URSS (N.S.)*, **18**, 405-407 (1938).

<sup>7</sup> Banach, *loc. cit.*, p. 80.

<sup>8</sup> Mazur, S., "Über konvex Mengen in linearen normierten Räumen," *Studia Math.*, **4**, 70-84 (1933).

<sup>9</sup> See Kakutani, S., "Weak Topology and Regularity of Banach Spaces," *Proc. Imp. Acad. Tokyo*, **15**, 169-173 (1939).

<sup>10</sup> *Proof:* If  $X$  is a limit point of  $M$  in  ${}_\tau E^{**}$  and  $(f_1, \dots, f_n)$  are arbitrary elements of  $E$ , there exists a sequence  $(x_m)$  in  $M$  such that  $|X(f_i) - f_i(x_m)| < 1/m$  ( $i = 1, \dots, n$ ). A standard compactness argument yields an element  $x$  in  $M$  such that  $X(f_i) = f_i(x)$  ( $i = 1, \dots, n$ ). But then  $X$  lies in  $E$ , and hence in  $M$ .

<sup>11</sup> See Alaoglu, L., and Birkhoff, G., "General Ergodic Theorems," *Ann. Math.*, **41**, 293-309 (1940).



## NOTE ON THE CRITICAL POINTS OF HARMONIC FUNCTIONS

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Two theorems recently proved by the writer have wide application in the study of the location of critical points of (a) harmonic measure, and (b) Green's functions for multiply connected regions of the extended plane, as we wish here to indicate. The present method involves primarily the conformal map onto the interior of a circle of the universal covering surface  $R^\infty$  of a given region  $R$ , a method used extensively by R. Nevanlinna in the study of harmonic measure.

The first theorem<sup>1</sup> to be applied is

**THEOREM 1.** *Let  $C$  be the unit circle in the  $w$ -plane, and let  $R$  be the interior of  $C$  with the deletion of closed regions bounded by the mutually exterior and disjoint Jordan curves  $C_1, C_2, \dots, C_m$  interior to  $C$ . Let  $\omega(w, C_1 + \dots + C_m, R)$  denote the harmonic measure of the set  $C_1 + \dots + C_m$  in the point  $w$  of  $R$  with respect to  $R$ , that is to say, this function  $\omega$  is harmonic and bounded interior to  $R$ , and takes on continuously the boundary values zero on  $C$  and unity on  $C_1 + \dots + C_m$ . Then the critical points interior to  $R$  of  $\omega(w, C_1 + \dots + C_m, R)$  lie in the smallest non-euclidean convex region in  $C$  containing  $C_1 + \dots + C_m$ .*

The non-euclidean lines interior to  $C$  are, of course, the arcs of circles orthogonal to  $C$ .

We shall use Theorem 1 in the proof of

**THEOREM 2.** *Let  $R$  be a region bounded by the disjoint Jordan curves  $C_1, C_2, D_1, \dots, D_m$ , and let  $S$  be the doubly connected region bounded by  $C_1$  and  $C_2$ . Then for constant  $\mu$ , any region  $\omega(z, C_1, S) > \mu \geq 1/2$  or  $\omega(z, C_2, S) > \mu \geq 1/2$  which contains no point of  $D = D_1 + D_2 + \dots + D_m$  contains no critical point of  $\omega(z, D, R)$ .*

We map conformally the universal covering surface  $S^\infty$  of  $S$  onto a strip bounded by two parallel straight lines, the latter corresponding to  $C_1$  and  $C_2$ , respectively. Each curve  $D_k$  is mapped into infinitely many Jordan curves  $D_{k1}, D_{k2}, \dots$ , successive curves of which are congruent under a suitable translation independent of  $k$  parallel to the sides of the strip. Adjacent to the sides of the original strip are then new strips parallel to them free from points of the images of  $D$ .

Map now the parallel strip which is the image of  $S^\infty$  onto the interior of the unit circle  $C$  in the  $w$ -plane. The ends of the strip correspond to two points  $A$  and  $B$  of  $C$ ; each line parallel to the sides of the original strip corresponds to a circular arc through  $A$  and  $B$ . Denote by  $C_1'$  and  $C_2'$  the arcs of  $C$  terminated by  $A$  and  $B$  which are the images of  $C_1$  and  $C_2$ , respectively, counted infinitely often. A certain closed lens-shaped region

$L$  interior to  $C$  bounded by circular arcs terminating in  $A$  and  $B$  contains all the images  $D'_{kq}$  of the curves  $D_k$ ; choose  $L$  as the smallest such region containing also the circular arc  $AB$  orthogonal to  $C$ . Then  $L$  is non-euclidean convex, and is bounded by arcs  $\omega(w, C_1', |w| < 1) = \mu_1 \geq 1/2$ ,  $\omega(w, C_2', |w| < 1) = \mu_2 \geq 1/2$ . It is not difficult to show that  $\omega(z, D, R)$  as interpreted in the  $w$ -plane is on any closed set in  $C$  exterior to  $L$  the uniform limit of the harmonic measure of any finite set  $\sum D_{kq}'$  with respect to the interior of  $C$  less the closed interiors of that set  $\sum D_{kq}'$ . Since  $L$  contains all the curves  $\sum D_{kq}'$ , it contains all critical points of this variable harmonic function, and hence  $L$  contains all critical points of the limit  $\omega(z, D, R)$ .

The function  $\omega(w, C_j', |w| < 1)$  is automorphic under any map of  $|w| < 1$  onto itself which leaves the image of  $S$  invariant, and is single-valued on  $S$ , so we have  $\omega(w, C_j', |w| < 1) = \omega(z, C_j, S)$ , for the properties of the harmonic measure define the function uniquely. Theorem 2 now follows at once.

The conclusion of Theorem 2 applies equally well to the location of the critical points of the complementary harmonic measure  $\omega(z, C_1 + C_2, R) = 1 - \omega(z, D, R)$ .

In the special case that  $C_1$  and  $C_2$  are circles—and this case can always be obtained by a suitable conformal map of  $S$ —the loci  $\omega(z, C_j, S) = \text{const.}$  are circles of the coaxial family determined by the circles  $C_1$  and  $C_2$ ; the locus  $\omega(z, C_j, S) = 1/2$  is the circle with respect to which  $C_1$  and  $C_2$  are mutually inverse. In this case Theorem 2 is especially simple to apply.

We leave to the reader the details of the proof of a limiting case of Theorem 2:

**THEOREM 3.** *Let  $R$  be a doubly connected region bounded by the Jordan curves  $C_1$  and  $C_2$ . Then any region  $\omega(z, C_1, R) > \mu \geq 1/2$  or  $\omega(z, C_2, R) > \mu \geq 1/2$  not containing the point  $z_0$  of  $R$  fails to contain the critical point of Green's function  $g(z, z_0, R)$  for  $R$  with pole in  $z_0$ .*

The general method of proof of Theorem 2 applies also if  $R$  is bounded by disjoint Jordan curves  $C_1, C_2, \dots, C_n, D_1, D_2, \dots, D_m$ , and we wish to study the location of the critical points of  $\omega(z, D, R)$ , where  $D = D_1 + D_2 + \dots + D_m$ . The case  $n = 1$  can be treated by a smooth map of the interior of the circle  $C$  of Theorem 1. In the case  $n > 2$ , as in the case  $n = 2$ , we map onto the interior of  $C: |w| = 1$  the universal covering surface  $S^\infty$  of the region  $S$  bounded by  $C_1, C_2, \dots, C_n$ . In the present case the image of  $C_k$  is not one single arc of  $C$ , but fills an infinite number of distinct arcs of  $C$  which are separated by arcs of  $C$  which are images of the other curves  $C_j$ . The harmonic measure  $\omega(w, C_k', |w| < 1)$ , where  $C_k'$  is a single arc of  $C$  whose points correspond to points of  $C_k$ , is not identical with  $\omega(z, C_k, R)$ ; the function  $\omega(w, C_k', |w| < 1)$  is automorphic under some but not all substitutions of the group of automorphisms of the function

which maps  $|w| < 1$  onto  $S^\infty$ . The set of images  $D'$  of all the curves  $D_k$  lies interior to  $C$ , and has as limit points on  $C$  an infinity of points, the complement of the set of arcs of  $C$  (of total length  $2\pi$ ) corresponding to the curves  $C_k$ . Denote by  $R'$  the image of  $R$  interior to  $C$ , and by  $\bar{R}'$  its closure. Then the function  $\omega(z, D, R) = \omega(w, D', R')$  is the limit of the harmonic measure in the point  $w$  with respect to the region  $|w| < 1$  less the closed interiors of a finite number of the curves  $D_k'$  which are the images of the  $D_k$ , of that finite number of curves  $D_k'$ ; this limit is uniform on any closed point set in  $\bar{R}'$  containing no limit point of  $D'$  on  $C$ . Consequently, by Theorem 1 no critical point of  $\omega(w, D', R')$  lies in a region  $\omega(w, C_k', |w| < 1) > \mu \geq 1/2$  which contains no point of  $D'$ , where  $C_k'$  indicates not the total image of  $C_k$  on  $C$  but a single arc of  $C$  whose points correspond to  $C_k$  traced infinitely many times. Otherwise expressed, all critical points of  $\omega(w, D', R')$  lie in any non-euclidean convex region interior to  $C$  which contains all points of  $D'$ . It is to be noted that non-euclidean geometry defined interior to  $C$  enables us to define non-euclidean geometry in  $S$ , and that the latter definition is independent of the particular conformal map chosen to map  $S^\infty$  onto  $|w| < 1$ . We state

**THEOREM 4.** *Let  $R$  be a region bounded by the disjoint Jordan curves  $C_1, C_2, \dots, C_n, D_1, D_2, \dots, D_m$ , and let  $S$  be the region containing  $R$  bounded by  $C_1, \dots, C_n$ . Let  $S^\infty$  denote the universal covering surface of  $S$ , and define non-euclidean lines on  $S$  by the conformal map of  $S^\infty$  onto the interior of the circle  $|w| = 1$ . Then any non-euclidean convex region of  $S$  which contains all the points of  $D = D_1 + \dots + D_m$  also contains all critical points of  $\omega(z, D, R)$ . Otherwise expressed, if  $C_k'$  is an arc of  $|w| = 1$  whose points correspond to points of  $C_k$ , then any region  $\omega(w, C_k', |w| < 1) > \mu \geq 1/2$  which contains no image point of  $D$  contains no critical points of  $\omega(z, D, R)$ .*

The conclusion of Theorem 4 applies also to the critical points of  $\omega(z, C_1 + \dots + C_n, R) = 1 - \omega(z, D, R)$ .

A limiting case of Theorem 4 can be proved from Theorem 4 itself:

**THEOREM 5.** *Let  $R$  be a region bounded by the disjoint Jordan curves  $C_1, C_2, \dots, C_n$ ; let  $R^\infty$  denote the universal covering surface of  $R$ , and define non-euclidean lines on  $R$  by mapping  $R^\infty$  conformally onto the interior of the circle  $|w| = 1$ . Then any non-euclidean convex region of  $R$  which contains the point  $z_0$  of  $R$  also contains all critical points of Green's function  $g(z, z_0, R)$  for  $R$  with pole in  $z_0$ . Otherwise expressed, if  $C_k'$  is an arc of  $|w| = 1$  whose points correspond to points of  $C_k$ , then any region  $\omega(w, C_k', |w| < 1) > \mu \geq 1/2$  which contains no image of  $z_0$  contains no critical points of  $g(z, z_0, R)$ .*

Theorems 3 and 5 extend at once to a sum

$$g(z, z_0, R) + g(z, z_1, R) + \dots + g(z, z_n, R)$$

of Green's functions. In this extension of Theorem 3, if all the points  $z_k$  lie on  $\omega(z, C, R) = 1/2$ , so do all critical points of this sum.

The situation of Theorem 4 can be studied by other methods, namely, the conformal map onto a circle of the universal covering surface of the region containing  $R$  bounded by any particular subset of the curves  $C_1, C_2, \dots, C_n, D_1, D_2, \dots, D_m$ . We consider in detail the mapping of the universal covering surface  $R^\infty$  of  $R$ . Here we need the following theorem:

**THEOREM 6.** *Let  $\alpha_1, \alpha_2, \dots, \beta_1, \beta_2, \dots$  be two sets of mutually non-overlapping open arcs of the unit circle  $C: |z| = 1$ , of total length  $2\pi$ ; we use the notation  $\alpha = \alpha_1 + \alpha_2 + \dots, \beta = \beta_1 + \beta_2 + \dots$ . Then no critical points of  $\omega(z, \alpha, |z| < 1)$  lie in either of the regions  $\omega(z, \alpha_k, |z| < 1) > 1/2, \omega(z, \beta_k, |z| < 1) > 1/2$ . If  $\alpha_j$  and  $\beta_k$  have an end-point in common, no critical points of  $\omega(z, \alpha, |z| < 1)$  lie in the region  $\omega(z, \alpha_j + \beta_k, |z| < 1) > 1/2$ .*

In Theorem 6, two arcs  $\alpha_j$  and  $\alpha_k$  having an end-point in common can be considered to form a single arc  $\alpha_e$  without altering  $\omega(z, \alpha, |z| < 1)$  or the conclusion. The conclusion of Theorem 6 obviously applies also to the critical points of  $\omega(z, \beta, |z| < 1) = 1 - \omega(z, \alpha, |z| < 1)$ .

We omit the proof of Theorem 6; the proof can be given from that of the corresponding theorem<sup>2</sup> concerning a finite number of arcs  $\alpha_k$  and  $\beta_k$ . We find at once

**THEOREM 7.** *Let  $R$  be a region bounded by the disjoint Jordan curves  $C_1, C_2, \dots, C_n, D_1, D_2, \dots, D_m$ . Set  $C = C_1 + C_2 + \dots + C_n, D = D_1 + D_2 + \dots + D_m$ . Let  $R^\infty$  denote the universal covering surface of  $R$ , which we map onto the interior of  $\Gamma: |w| = 1$ . Then no critical points of  $\omega(z, D, R)$  lie in either of the regions  $\omega(w, \alpha_k, |w| < 1) > 1/2$ , or  $\omega(w, \beta_k, |w| < 1) > 1/2$ , where  $\alpha_k$  is any arc of  $\Gamma$  whose points correspond to points of  $C$ , and where  $\beta_k$  is any arc of  $\Gamma$  whose points correspond to points of  $D$ .*

This conclusion also applies to the critical points of  $\omega(z, C, R) = 1 - \omega(z, D, R)$ . The conclusion can of course be expressed in terms of non-euclidean geometry in  $|w| < 1$ , on  $R^\infty$ , and in  $R$ .

The application of Theorem 7 is of particular interest if Carleman's Principle of Gebietserweiterung is used. Thus let  $\gamma$  be an arbitrary Jordan curve interior to  $R$  which intersects no  $C_k$  or  $D_k$  but separates  $C - C_1$  and  $D$  from  $C_1$ . Denote by  $S$  the region bounded by  $\gamma$  and  $C_1$ , and by  $S^\infty$  its universal covering surface. Then  $S^\infty$  lies on  $R^\infty$ , and in fact an infinity of replicas of  $S^\infty$  lie on  $R^\infty$ . If  $\alpha_1$  is a particular maximal arc of  $\Gamma$  which corresponds to  $C_1$  taken infinitely many times, then a replica of  $S^\infty$  corresponds to a region  $\rho_1$  interior to  $\Gamma$  bounded by  $\alpha_1$  and by an arc interior to  $\Gamma$  joining the end-points of  $\alpha_1$ . Consequently we have interior to  $\rho_1$

$$\omega(w, \alpha_1, |w| < 1) > \omega(w, \alpha_1, \rho_1),$$

so the region  $\omega(w, \alpha_1, \rho_1) > 1/2$  lies interior to the region  $\omega(w, \alpha_1, |w| < 1) > 1/2$ . In the map of  $S^\infty$  onto  $\rho_1$ , the function  $\omega(w, \alpha_1, \rho_1)$  is single-valued in  $S$ , and is equal to  $\omega(z, C_1, S)$ . Thus we have the

**COROLLARY.** *Under the conditions of Theorem 7, let  $\gamma$  be an arbitrary Jordan curve interior to  $R$  which intersects no  $C_k$  or  $D_k$  but separates  $C - C_1$  and  $D$  from  $C_1$ , and let  $S$  be the subregion of  $R$  bounded by  $C_1$  and  $\gamma$ . Then no critical point of  $\omega(z, D, R)$  lies in the region  $\omega(z, C_1, S) > 1/2$ .*

This Corollary is of especial interest when  $C_1$  and  $\gamma$  are circles, for in that case the locus  $\omega(z, C_1, S) = 1/2$  is also a circle. In any case, the Corollary yields for every curve  $C_k$  (or  $D_k$ ) an annular region bounded in part by that curve and which is free from critical points of  $\omega(z, D, R)$ .

Even if  $C_1$  and  $\gamma$  are not circles, suppose a circle  $\gamma'$  in  $S$  separates  $C_1$  and  $\gamma$ , and suppose a circle  $C_1'$  exterior to  $S$  is separated from  $S$  by  $C_1$ . Let  $S'$  denote the annular region bounded by  $C_1'$  and  $\gamma'$ . At a point  $z$  on  $\gamma'$  or on  $C_1$  we have  $\omega(z, C_1', S') < \omega(z, C_1, S)$ , so this inequality is valid at every point  $z$  common to  $S$  and  $S'$ . Consequently the locus  $\omega(z, C_1', S') > 1/2$ , if any, in  $S$  is free from critical points of  $\omega(z, D, R)$ .

Theorem 6 is of obvious significance in the study of the geometric situation of Theorem 7, but where we now study the harmonic measure of an arbitrary set of boundary arcs of  $R$ .

**THEOREM 8.** *Let  $R$  be a region bounded by a finite number of disjoint Jordan curves, and let  $A_1, A_2, \dots, A_n$  be a finite number of disjoint Jordan arcs of the boundary of  $R$ , with  $A = A_1 + A_2 + \dots + A_n$ . Let the images of the arcs  $A_1, A_2, \dots, A_n$  when  $R^\infty$  is mapped onto the interior of  $\Gamma: |w| = 1$  be the arcs  $\alpha_1, \alpha_2, \dots$ , and let the complementary arcs of  $\Gamma$  be  $\beta_1, \beta_2, \dots$ . Any non-euclidean line of the interior of  $\Gamma$  which joins the end-points of an  $\alpha_k$ , or the end-points of a  $\beta_k$ , or the extreme end-points of an arc  $\gamma$  of  $\Gamma$  consisting of an arc  $\alpha_k$  and an adjacent  $\beta_j$ —such a line separates no critical point of the harmonic measure  $\omega(w, \alpha_1 + \alpha_2 + \dots, |w| < 1)$  from the stated arc which it spans. That is to say, no critical point of this harmonic measure lies in the region  $\omega(w, \alpha_k, |w| < 1) > 1/2$ ,  $\omega(w, \beta_k, |w| < 1) > 1/2$ , or  $\omega(w, \gamma, |w| < 1) > 1/2$ . Thus no critical point of  $\omega(z, A, R)$  lies in a subregion of  $R^\infty$  bounded by a non-euclidean line of  $R^\infty$  and either an arc  $A_k$ , or an arc  $B_k$  of the set of arcs complementary to  $A$ , or an arc  $A_k + B_j$  consisting of an  $A_k$  plus one of the set of complementary arcs; no such critical point lies in any of the regions  $\omega(z, A_k, R^\infty) > 1/2$ ,  $\omega(z, B_k, R^\infty) > 1/2$ ,  $\omega(z, A_k + B_j, R^\infty) > 1/2$ .*

Theorem 8 defines a region of  $R$  adjacent to every  $A_k, B_k$ , and arc  $A_k + B_j$ , which is free from critical points of  $\omega(z, A, R)$ . In case one of the Jordan curves, say  $C_1$ , bounding  $R$  contains but a single one of the arcs  $A_k$ , say  $A_1$ , we prove that an entire neighborhood of  $C_1$  is thus defined which is free from critical points. Let  $B_1$  denote the arc of  $C_1$  complementary to  $A_1$ . An arc of  $\Gamma$  consists of the image of  $C_1$  traced in the same sense infinitely often, hence consists of an infinite number of arcs  $\alpha_{1k}$  which are images of  $A_1$  alternating with an infinite number of arcs  $\beta_{1k}$  which are images of  $B_1$ . Any such arc  $\alpha_{1k}$  can be paired with each of two adjacent arcs  $\beta_{1j}$ , to form an arc  $\gamma$  of Theorem 8; one of the two corresponding



non-euclidean lines on  $R^\infty$  commences and terminates at one end-point of  $A_1$ , while the other non-euclidean line on  $R^\infty$  commences and terminates at the other end-point of  $A_1$ ; together these two non-euclidean lines bound a neighborhood of  $C_1$  in  $R$  free from critical points. But this neighborhood of  $C_1$  is smaller than the neighborhood defined in Theorem 7 and considered in the study of the critical points of  $\omega(z, D, R)$ . Indeed, on  $\Gamma$  in Theorem 8 each arc  $\gamma$  is a *proper subset* of an arc of  $\Gamma$  which (used in Theorem 7) consists wholly of images of points of  $C_1$ ; the longer arc determines the larger subregion of  $|w| < 1$  free from critical points. Otherwise expressed, each region of Theorem 8 involving  $\gamma$  and free from critical points is defined as  $\omega(z, C_1, R^\infty) > 1/2$ , where  $C_1$  is counted as a bounding arc of  $R^\infty$  but once; the region free from critical points in Theorem 7 is defined as  $\omega(z, C_1, R^\infty) > 1/2$ , where  $C_1$  is repeated infinitely often as a boundary arc of  $R^\infty$ ; even in Theorem 7 we do not here take *all* points of  $C_1$  in every sheet of  $R^\infty$  in defining this harmonic measure.

The Principle of Gebietserweiterung applies also in the case of Theorem 8, where subregions are considered as defined on  $R^\infty$ . One special case is of fairly wide applicability:

**COROLLARY.** *Under the conditions of Theorem 8, let the arc  $E$  of the boundary of  $R$  consist of an arc  $A_k$ , or an arc  $B_k$  of the set of arcs complementary to  $A$ , or an arc  $A_k$  plus an adjoining arc  $B_k$ ; and let a circle (in the extended sense)  $\Omega$  be divided into arcs  $\Omega_1$  and  $\Omega_2$  by the end-points of  $E$ . Let  $E + \Omega_1$  bound a Jordan region in  $R$ , and let  $\Omega_2$  lie exterior to  $R$ . Then no critical point of  $\omega(z, A, R)$  lies in the subregion of  $R$  if any bounded by  $E$  and by the circular arc orthogonal to  $\Omega$  joining the end-points of  $E$ .*

<sup>1</sup> Walsh, J. L., *Bull. Amer. Math. Soc.*, 52, 346-347 (1946).

<sup>2</sup> Walsh, J. L., these PROCEEDINGS, 33, 18-20 (1948).

## THE PRODUCTION OF MUTATIONS IN STAPHYLOCOCCUS AUREUS BY IRRADIATION OF THE SUBSTRATE

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A knowledge of the gene including its chemistry, function and mutation is of primary importance in understanding life. Muller<sup>1</sup> (see his bibliography also) has summarized the vast collection of information available at present on these subjects. Nevertheless, despite the interest in these problems, little information exists as to the exact chemical nature of any particular gene, much less the differences that exist between genes and



alleles. One method of attack on the nature of the gene system is through a study of mutation. Until Muller<sup>2</sup> showed the effectiveness of x-radiation, no method of producing gene mutations was known. Later it was demonstrated that longer wave length of radiation including ultra-violet would also produce mutations. More recently Auerbach and Robson<sup>3</sup> have demonstrated that mutations may result from the application of certain chemicals (including mustard gas and allied substances).

There are several difficulties in the interpretation of the effect of radiation on the mutation process. First, x-radiation is so drastic a treatment that particular kinds of effects cannot be predicted to the exclusion of others. Second, mutation from irradiation has proved to be a random process. Third, although it has been proved that the mutation rate is directly proportional to the number of individual ionizations, it has not been possible to determine how much of the effect of irradiation resulted from a direct hit on a gene as against an indirect effect from a hit on some other substance in the cell.

Another line of attack has been to compare the effects of x-rays with ultra-violet irradiation, for certain important differences exist in their effects, Stadler.<sup>4</sup> The reasons for these differences have not been completely determined, because here again the material changed directly by the irradiation is unknown. The work on the induction of mutation by chemicals is as yet fragmentary. It should be emphasized that in none of this work has there been evidence for selective induction of particular gene mutations. All agents so far utilized have been *general*, and, therefore, make a study of the particular change involved in mutation exceedingly difficult. As a result there exists no definite knowledge of the chemical or physical change that accompanied any particular mutation.

This paper reports experiments combining irradiation and chemical production of mutations in bacteria. This was accomplished by allowing bacteria to reproduce in a medium containing known substances which had been subjected to irradiation, and then determining if mutations had been induced. The advantage of this method in the chemical analysis of the gene lies in the fact that particular known substances can be irradiated and their effect on gene mutation studied. In addition, the chemical and physical changes that have occurred in those irradiated substances which cause an increase in mutation rate can be investigated.

*Experimental.*—A 24-hour broth culture of *Staphylococcus aureus* (F.D.A. strain No. 209) was divided into two portions. One half was retained as a control and the other was placed in a petri dish and exposed for 6 minutes at a distance of 50 cm. to radiation produced by a Hanovia double-U SC-2537 ultra-violet mercury vapor lamp operating at 100 milliamperes. Both portions were then plated in appropriate dilutions in nutrient agar and in nutrient agar containing various concentrations of penicillin. After

incubation for 72 hours at 37°C. the colonies were counted. The results are tabulated under Exp. 1 in table 1. All plating was done in triplicate and the figures given are averages of the 3 plates. The control organisms show a distribution of resistant forms similar to that described by Demerec.<sup>5</sup> Although the radiation killed about 40% of the bacteria, a greater number of resistant organisms were present among the survivors than in the unirradiated controls. The exposure to the light produced resistant individuals that had not been present originally.

TABLE 1

INCREASE IN RATE OF MUTATION TO PENICILLIN RESISTANCE BY IRRADIATION OF THE BACTERIA OR THE MEDIUM IN WHICH THEY ARE GROWN

PENICILLIN CONCENTRATION, UNITS/ML.	PLATE COUNT			
	EXP. 1. BACTERIA IRRADIATED CONTROL	IRRADIATED	EXP. 2. MEDIUM IRRADIATED CONTROL	IRRADIATED
0	221,000,000	135,000,000	114,000,000	46,000,000
0.027	483,000	531,000	27,300	182,300
0.030	86,100	269,800	7,500	40,900
0.033	4,670	56,200	4,610	33,100
0.036	1,250	12,700	2,200	16,900
0.039	1,100	5,180	415	6,500
0.042	495	4,400	317	4,600
0.045	136	1,660	12	1,360

Such individuals are regarded as mutants which differ from the normal population in an alteration of one or several genes. Ultimately, this alteration must be resolved to chemistry and physics. If chemical changes in genes can be brought about by exposure to radiation the possibility is presented that such changes might be introduced into the building materials from which the genes are produced. Therefore, experiments were devised to determine the effect of the irradiation of the substrate upon the mutation rate of organisms subsequently inoculated therein.

Nutrient broth was exposed to radiation from the ultra-violet lamp previously mentioned. After a 3-hour exposure the broth was transferred to a culture flask and an unirradiated equal portion from the same batch of nutrient broth was transferred to a similar flask. Immediately both were inoculated in an identical manner, at the rate of about one million cells per ml. from a 24-hour culture of *S. aureus*. Both flasks were incubated for 5 hours and then appropriate dilutions were plated on nutrient agar containing concentrations of penicillin varying from 0 to 0.045 Oxford units per ml. In all experiments a sufficiently large flask of nutrient agar of each penicillin concentration was prepared so that the plates of both the irradiated and unirradiated series would be poured with agar from the same flask. This eliminates the possibility of errors in dosage at any single level when comparisons are made within any experiment. The plates were counted after incubation for 72 hours at 37°C. and the averages from the triplicate platings are presented as Exp. 2 in table 1.

It is evident that neither culture had attained maximum growth in the 5 hours which had elapsed before plating. The culture growing in the irradiated broth had produced only 46 million cells or about  $5\frac{1}{2}$  generations, while the culture growing in the control broth produced 114 million cells or about 7 generations. In spite of this lower total production of cells the actual number of resistant bacteria in the irradiated broth at each level of penicillin is many times greater. This fact helps to rule out selection as the cause of the phenomenon and suggests that we are dealing with induced mutation.

This experiment was repeated many times with a number of variations. The penicillin routinely employed was obtained from Commercial Solvents, but Abbott and Merck penicillins were also used. Another strain of *S. aureus* was substituted for the F.D.A. strain in a few of the experiments. For many of the tests a Hanovia analytical model quartz-mercury lamp with Type A burner was used at a distance of 20 cm. and the irradiation of the broth was continued for only 90 minutes. In most cases water lost from the broth by evaporation during exposure to the lamp was replaced, but in some cases it was not. The size of inoculum was varied and the time of plating was delayed to as long as 30 hours after inoculation. Although some quantitative differences were noted the results obtained in all cases were qualitatively similar, *viz.*, cultures grown in irradiated broth produced more mutations.

TABLE 2

INCREASE IN MUTATION RATE BY IRRADIATION OF COMPONENTS OF A SYNTHETIC MEDIUM

PENICILLIN CONCENTRATION, UNITS/ML.	PLATE COUNTS ON CULTURES GROWN IN A SYNTHETIC MEDIUM AFTER IRRADIATION OF THE INDICATED COMPONENTS			
	NONE (CONTROL)	COMPLETE MEDIUM	MINERAL SALTS	AMINO ACIDS AND VITAMINS
0	800,000,000	No growth	900,000,000	195,000,000
0.04	33,000	No growth	40,000	150,000
0.07	4	No growth	4	196
0.10	1	No growth	1	16

If the synthetic medium described by Fildes, *et al.*,<sup>6</sup> is subjected to the same irradiation as the nutrient broth in Exp. 2, table 1, the organisms make no growth. This medium consists of mineral salts, the amino acids found in casein, glucose, and the vitamins thiamin, niacin and biotin. In our experiments we supplemented the latter with one mg. per liter each of adenine, guanine and uracil. Each component was prepared in sterile solution at several times the final concentration so that it could be irradiated separately before combining with the other constituents of the medium. In the experiment reported in table 2, four batches of the synthetic medium were prepared: (1) an unirradiated control, (2) the complete medium irradiated, (3) a double strength solution of the mineral salts irradiated and then added to the remaining components, (4) a triple strength

solution of the amino acids and vitamins irradiated and added to the remaining components. The four media were inoculated in an identical manner and incubated for 24 hours. At that time the culture in which all the components were irradiated had made no visible growth so plate counts were made only on the other three cultures. Irradiation of the mineral salts resulted in a mutation rate no higher than the control but irradiation of the amino acids and vitamins resulted in a marked increase in the mutation rate. Further experiments showed that a good part of the toxic effect (which prevented growth when the complete synthetic medium was subjected to the amount of radiation routinely used in nutrient broth experiments) arose from the glucose. When the irradiation of the glucose was decreased to a level that permitted growth, the mutation rate of cultures grown in media prepared from it was not enhanced.

That the irradiation of the amino acids alone increased the mutation rate is indicated in table 3. Nutrient broth cultures were included in this experiment because the plating was done on agar containing streptomycin as well as on agar containing penicillin. It is evident that rate of mutation

TABLE 3  
INCREASE IN THE RATE OF MUTATION TO STREPTOMYCIN AND PENICILLIN RESISTANCE

INHIBITOR, UNITS/ML.	NUTRIENT BROTH		SYNTHETIC MEDIUM	
	CONTROL	IRRADIATED	CONTROL	AMINO ACIDS IRRADIATED
0	300,000,000	260,000,000	1,250,000,000	900,000,000
Penicillin				
0.04	13,000	120,000	12,000	55,000
0.07	10	310	30	1,520
Streptomycin				
1.0	42,000	140,000	30,000	168,000
3.0	5,000	33,000	2,700	23,000

to streptomycin resistance follows the same pattern observed with penicillin resistance. The streptomycin employed in the experiment was crystalline material obtained from Merck which had a potency of 187 units per mg. Other experiments were carried out with lyophilized powder from Commercial Solvents put up in ampules containing 100,000 units.

To demonstrate that the streptomycin- and penicillin-resistant organisms were the result of different mutations, a number of colonies of the mutants were picked and transferred to nutrient agar. Similarly, a number of subcultures were isolated from the control plates. After 24-hour incubation a uniform inoculum from each isolate was streaked to sectors of plates containing streptomycin or penicillin. The plates were incubated for 48 hours and the growth of each culture on each concentration of antibiotic was noted (table 4). Due to the small inoculum used the results were generally clear-cut, although occasionally only a few colonies appeared;

these were reported as positive growth. It will be observed that mutants selected for penicillin resistance differ greatly from the controls when tested on penicillin agar but are very similar to the control organisms when tested on streptomycin agar. The streptomycin-resistant mutants differ from both the controls and the penicillin-resistant mutants. The mutant population is composed of different individuals whose gene differences are induced during growth in the irradiated substrate; the specific mutants are then separated from each other and from the normal population by plating on agar containing the inhibiting substances.

After the specificity of the resulting mutants was demonstrated, attempts were made to determine if specificity could be introduced into the induction of mutation, i.e., if the rate of mutation of one particular gene could be influenced without affecting the rate of mutation of others. Preliminary experiments indicate that this may be possible, for by suitable irradiation procedures we have been able to increase the rate of mutation to penicillin resistance without affecting the rate of mutation to streptomycin resistance.

TABLE 4  
SPECIFICITY OF THE DRUG-RESISTANT MUTANTS

STRAINS	NO. TESTED	NO. OF STRAINS GROWING ON MEDIUM CONTAINING:						
		PENICILLIN (UNITS/ML.)				STREPTOMYCIN (UNITS/ML.)		
		0.05	0.1	0.15	1.0	3	5	10
Penicillin-resistant	21	21	15	9	1	14	6	1
Controls	24	13	1	0	0	12	7	2
Streptomycin-resistant	29	12	2	0	0	29	29	24

*Discussion.*—These data are the preliminary results of the new method of investigation of gene mutation and gene chemistry. Irradiation is used to *activate* selected chemicals, which, when utilized by the cells, cause mutations. These substances might be termed *activated mutators*. The experimental methods used are very effective in detecting mutations to resistance to toxic agents, in this case penicillin and streptomycin.

Several lines of evidence indicate that these are induced mutations, not the result of selection:

1. In an immature culture there are numerically many more mutants in the irradiated broth despite a smaller total population (table 1).

2. When mutant strains were isolated and their rate of growth was followed in both irradiated and unirradiated broth it was found that they grew more slowly in the former; the rate of growth in both media did not differ significantly from that of control organisms.

3. These mutations are of independent origin and do not represent a general increase in resistance to toxic agents for, on testing, the penicillin-



resistant mutants are no more resistant to streptomycin than are the controls and vice versa (table 4).

It seems improbable that this method of treatment is limited to the production of mutations concerned with resistance. The first two things tried—mutations to resistance to penicillin and streptomycin—were induced by this treatment. With suitable tests many other types of mutation should be detected. Therefore, we believe this will prove to be a generally useful procedure, within its limitations, when used to produce other types of mutation, and mutations in other organisms.

Little need be said of the obvious advantage of this method for the study of mutation and gene chemistry. Instead of treating an exceedingly heterogeneous living organism, we are treating a selected chemical and determining if it produces mutations. If it does, we can investigate the physical or chemical changes that have occurred. These may be either or both of the following: (1) The production of different chemical compounds under the influence of irradiation. (2) Some mechanism involving a shift to a higher energy level by the absorption of a quantum of energy and subsequent effects of this energy transfer. At present we cannot decide between these alternatives, although we have determined that the mutating ability of the treated material is reduced on aging and more rapidly by heat treatment. This suggests that alternative (2) may be correct, but of course it only concerns these particular mutations. On the other hand, it is known that a definite chemical substance, nucleic acid, can induce transformation in the genetic material of microorganisms (Avery, *et al.*<sup>7</sup>) The possibility of producing selected mutations offers a fascinating field for further investigation. Certainly the possibility is inherent in this method. Genes must differ; therefore, we may be able to select agents which will affect only one or at most a few genes and so, indirectly, study gene chemistry. In the experiments reported here, we were able to produce two types of resistant mutants. The difference in survival in the different concentrations of the antibiotics indicates that several different alleles or different mutations are involved (tables 1, 2, 3). We have not been able to determine if the treatment produces changes in an existing gene or if it causes an error in its autocatalytic reproduction. Under ordinary circumstances genes are remarkably free from errors in copy as exemplified by the low rate of spontaneous mutation.

There are several general considerations which follow from these discoveries. For example, a study of the effect of irradiated substrate on the development of cancerous tissue may prove profitable. If any of the activated mutators prove stable this phenomenon must be considered in nutrition and in safeguarding the use of atomic energy. The most important consideration is the possibilities opened for further investigation into the gene and mutation. These experiments have shown that at least part of



the mutations produced by irradiation may be the result of an indirect effect in addition to those from a direct hit on the gene. They may explain at least part of the discrepancy between the observed normal mutation rate and that which has been calculated as attributable to direct hits on genes, Muller.<sup>1</sup> Natural radiation is much more important in the mutation process if it can induce mutation by an effect on the food as well as on the organism.

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<sup>1</sup> Muller, H. J., *Proc. Roy. Soc.*, **134**, 1-37 (1947).

<sup>2</sup> Muller, H. J., *Science*, **66**, 84-87 (1927).

<sup>3</sup> Auerbach, C., and Robson, J. M., *Nature*, **154**, 81 (1944).

<sup>4</sup> Stadler, L. J., *Cold Spring Harbor Symposia on Quantitative Biology*, **9**, 168-178 (1941).

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<sup>6</sup> Fildes, P., Richardson, G. M., Knight, B. C. J. G., and Gladstone, G. P., *Brit. J. Exp. Path.*, **17**, 481 (1936).

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*ON THE DISTRIBUTION OF TITANIUM OXIDE AND CARBON  
STARS IN OUR PART OF THE MILKY WAY*

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Communicated March 3, 1947

The Dearborn Observatory Survey of Faint Red Stars from four and a half degrees below the equator to the north pole has been completed. Some 44,000 stars of types K5, M0 to M9, R and N have been classified, and their magnitudes have been determined. Publication of the final half of these stars, and of a discussion of them all is under way.

In each of 317 areas magnitude limits ranging from 11 to nearly 13 were reached.

Upon plotting the K5 and M stars in galactic coördinates several items became apparent.

1. The obscuring clouds of interstellar material certainly affect the distribution decidedly. In fact, the axis of maximum frequency makes an angle of about 14 degrees with the galactic equator crossing it from south to north at longitude  $90^\circ$ . Other characteristics of distribution indicate the possibility that reasons, more basic than obscuration by cosmic clouds, may exist.

2. Among the 44,000 stars, 2894 belong in the advanced titanium oxide classes, M5 to M9. Of these, 1684 have apparent magnitudes brighter than 10 and only 1210 are of magnitude 10 or fainter. Dwarfs at distances from 100 to 1000 light years are too scarce to be statistically important in the observed frequencies. We are dealing mostly with giants, and beyond galactic latitudes of 30 degrees north and south they are nearly all in the brighter group. This survey has reached the galactic boundaries in these directions.

Along the broad galactic band we expected to find great numbers of distant red giants of these types recorded on our plates as faint stars. However, the ratio of numbers in the two magnitude groups is about 1, whereas the general stellation is 2 to 4 or more in favor of the fainter stars.

Stars of types M5 to M9 are the easiest of all to detect and classify on our red sensitive plates. We probably missed very few in the magni-

tude range from 7 to 13. We noticed this preponderance of bright giants from the start and have mentioned it before. We are now, on the basis of all of our material, obtained systematically in the northern 54 per cent of the sky, forced to conclude that spectra showing the TiO band are particularly abundant in our part of the Milky Way and perhaps that man has appeared upon the cosmic scene in an era when many stars in his vicinity are going through their "titanium oxide stage."

In the course of this survey, most of the known carbon stars in our zones have been reobserved and 209 new ones have been catalogued. Of the latter we feel certain about 144-89 of type N and 55 of type R. The total number of carbon stars now known in the whole sky is about 418-282 of type N and 136 of type R. The galactic concentration of both kinds is very high.

As early as in 1941 we reported the discovery of a nest of N stars just east of Orion. Including all of these stars now known this cluster stands out very clearly. In an area of 750 square degrees, roughly pentagonal in shape, centering in galactic longitude  $168^\circ$ , latitude  $-5^\circ$ , there are about 50 N stars, with only 2 R stars involved. On both sides of this nest along the galactic equator the two types are well mixed in frequency.

In Sanford's figure [*Astrophysical Journal*, 99, 156 (1944)] this cluster is noticeable. The average magnitude of carbon stars in his list is about 9. Ours average around 11. Since R stars are about 2 magnitudes less luminous than the N stars a thin cloud of obscuring material in the foreground might conceivably blot out the former while allowing the latter stars to shine through. This does not explain the presence of the N star nest and would probably make it even more pronounced, actually, than we have observed it.

We seem to be driven to the conclusion that bodies containing an excess of carbon are relatively very abundant in the region of this nest of N stars.

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## THE SPREAD OF MEASLES IN THE FAMILY

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Some years ago, with the help of several collaborators, I discussed Major Greenwood's theory of the spread of measles within the family.<sup>1</sup> That theory had two postulates: (1) if a primary case (or several co-primary cases) brought measles into a family in which there were  $m$  other susceptibles, the chances that  $m, m - 1, \dots, 0$  secondaries would result

would be  $p^m, mp^{m-1}q, \dots, q^m$  as though the children were independently infected, the chance of infection being  $p$ ; and (2) the secondary case or cases would expose the remaining susceptibles to infection for a subsequent generation of cases secondary to the secondaries (tertiary cases as we called them) with the same chance  $p$ . Greenwood had tested his theory against observation by comparing the observed and expected numbers of families which had 0, 1, 2,  $\dots$  cases *altogether* beyond the primary and had found a good fit. On the same gross comparison we also found a good fit to his theory with our very different data, but when we broke down the data into successive generations as called for by the detailed hypotheses (1) and (2), we found that (1) the calculated distribution of the numbers of families by numbers of cases in each generation did not fit the observations; and (2) the tertiary attack rate was significantly lower than the immediate or direct secondary attack rate. So the theory became untenable for our Providence data. I wish to examine the matter a trifle further.

Tabulation of the samples II (p. 446), IV (p. 447), and VI (p. 448) of families containing two susceptibles according as the families have 0, 1, 2 immediate secondary cases gives:

CASES	0	1	2	TOTAL
For II	34	61	239	334
For IV	15	41	129	185
For VI	49	102	368	519

The  $\chi^2$ -test shows that II and IV differ only about as much as might be expected by chance and may therefore be combined into VI without hesitation. The chance of a secondary case (derived from a primary) is  $p = 0.807$  and the chance distribution of the 519 families would be 19, 162, 338 instead of 49, 102, 368. The hypothesis (1) that measles spreads in the family although the children were independently infected is therefore untenable.

One way to express dependence of elements is to compute the number which would be required by the theory of chance to explain the observed standard deviation. The actual secondary attack rates in the families with 0, 1, 2 secondary cases are 0, 0.5, 1.0, respectively; their mean is 0.807 and their standard deviation squared (variance) is 0.016. If this be equated to  $pq/n$ , we find  $n = 1.46$ . Thus the two susceptibles in the family are behaving relative to contracting or escaping the infection as though they were about one and one-half.<sup>2</sup>

Another, and presumably better, method is to make an analysis free from the hypothesis that the chances of incidence within the family are independent. Let the two susceptibles in each family be designated in some way as  $A$  and  $B$  (for example,  $A$  may be the older and  $B$  the younger). Then the families may be entered in a 4-fold table according as  $A$  and  $B$  or  $A$  but not  $B$  or  $B$  but not  $A$  or neither  $A$  nor  $B$  contracts the disease.

In the notation of Yule, using parentheses to denote the numbers of cases with specified attributes, we have the table:<sup>3</sup>

	$A$	$\alpha$	
$B$	$(AB)$	$(\alpha B)$	$(B)$
$\beta$	$(A\beta)$	$(\alpha\beta)$	$(\beta)$
	$(A)$	$(\alpha)$	$N$

$$p_A = (A)/N, q_A = (\alpha)/N,$$

$$p_B = (B)/N, q_B = (\beta)/N.$$

By the theory of probabilities, when events are not independent, the chance of  $A$  and  $B$  is the chance of  $A$  multiplied by the chance of  $B$  if  $A$  occurs, or

$$p_{AB} = \frac{(AB)}{N} = \frac{(A)}{N} \cdot \frac{(AB)}{(A)}, \quad p_{\alpha\beta} = \frac{(\alpha\beta)}{N} = \frac{(\alpha)}{N} \cdot \frac{(\alpha\beta)}{(\alpha)}.$$

The probabilities  $p_{AB}$  and  $p_{\alpha\beta}$  are observable as both or neither without specification of the susceptibles as  $A$  and  $B$ , but without such specification we cannot determine  $(A\beta)$  and  $(\alpha B)$  though we here can determine the chance  $p_1$  of just one case and also the secondary attack rate  $s$  as

$$p_1 = \frac{(A\beta) + (\alpha B)}{N} \quad \text{and} \quad s = p_{AB} + \frac{1}{2} p_1.$$

If we will introduce  $\eta = p_A - p_B$ , the difference between the chances of  $A$  and of  $B$ , we may solve for all elements in terms of the observed quantities  $p_{AB}$ ,  $p_{\alpha\beta}$ ,  $p_1$ ,  $s$  and the hypothetical quantity  $\eta$  as follows:

$$\text{Chance of } A \text{ is } p_A = s + \frac{1}{2} \eta, \text{ chance of } B \text{ is } p_B = s - \frac{1}{2} \eta,$$

$$\text{Chance of } B \text{ if } A \text{ is } \frac{p_{AB}}{s + \frac{1}{2} \eta}, \text{ chance of } \beta \text{ if } \alpha \text{ is } \frac{p_{\alpha\beta}}{s - \frac{1}{2} \eta}$$

It may be observed that, in a general association table,  $\eta = p_A - p_B = p_{AB} - p_{\alpha\beta} = (A\beta)/N - (\alpha B)/N$ , may range from  $(A\beta)/N$  if  $(\alpha B)$  happens to vanish to  $-(\alpha B)/N$  if  $(A\beta)$  happens to vanish.

In sample VI, there is no way to distinguish the two susceptibles and we may assume that they are in fact equivalent (as they probably would not be if distinguished by ages), that is  $(A\beta)/N = (\alpha B)/N = 51/519 = 0.0982$  and  $\eta = 0$ . Then the secondary attack rate is 0.807, but in case one be assumed to be attacked the chance that the other will also be attacked is 0.88; and the escape rate is 0.193, but in case one be assumed to escape the chance that the other escape<sup>4</sup> is 0.49.

If we turn next to samples IX and XI (p. 450) with three susceptibles in addition to the primary, we have for immediate secondaries

CASES	0	1	2	3	TOTAL
For IX	4	11	18	67	100
For XI	4	16	8	27	55

The two samples differ enough so that it is very doubtful whether they may safely be combined into one (XIII). The secondary attack rate in IX is  $s = 0.827$  and in XI is  $s = 0.685$ . If we compute the variances of the attack rates  $p = 0, \frac{1}{3}, \frac{2}{3}, 1$  in the families, we find for IX and XI, respectively, 0.079 and 0.119; the respective values of  $pq$  are 0.143 and 0.216, and the value of the number of independent susceptibles turns out in either case to be  $n = 1.8$  which is well below 3.

An analysis can be made in terms of probabilities. It may be supposed that the three susceptibles are distinguished as possessing attributes  $A, B, C$ . There are then the three probabilities<sup>6</sup>

$$p_A = \frac{(A)}{N}, p_{B \cdot A} = \frac{(AB)}{(A)}, p_{C \cdot AB} = \frac{(ABC)}{(AB)},$$

namely, the chance of  $A$ , the chance of  $B$  if  $A$ , the chance of  $C$  if  $A$  and  $B$ , and many others, where the number in a class possessing assigned attributes is divided by the number in a class in which one of those attributes is missing, for example  $(A\beta\gamma)/(A\beta)$  which is "the chance of not- $C$  if  $A$  and not- $B$  be given." The general analysis would be needed in case the attributes (such as serial order in age) were assigned; for our data where there is no such assignment the only solution we shall seek is that for which the attributes are equivalent, i.e.,  $(A) = (B) = (C)$ , etc.

Under the assumption of equivalence we find

For IX:	$(ABC) = 67,$ $(\alpha\beta\gamma) = 4,$	$(AB\gamma) = (A\beta C) = (\alpha BC) = 6,$ $(A\beta\gamma) = (\alpha B\gamma) = (\alpha\beta C) = 11/3;$	
For XI:	$(ABC) = 27,$ $(\alpha\beta\gamma) = 4,$	$(AB\gamma) = (A\beta C) = (\alpha BC) = 8/3,$ $(A\beta\gamma) = (\alpha B\gamma) = (\alpha\beta C) = 16/3.$	
For IX:	$p_A = 0.827,$ $p_\alpha = 0.173,$	$p_{B \cdot A} = 0.88,$ $p_{\beta \cdot \alpha} = 0.44,$	$p_{C \cdot AB} = 0.92,$ $p_{\gamma \cdot \alpha\beta} = 0.52;$
For XI:	$p_A = 0.68,$ $p_\alpha = 0.32,$	$p_{B \cdot A} = 0.79,$ $p_{\beta \cdot \alpha} = 0.54,$	$p_{C \cdot AB} = 0.91,$ $p_{\gamma \cdot \alpha\beta} = 0.43.$

It is seen that the chance of an additional direct secondary case increases with the number of cases already assumed among the susceptibles in either sample; but the chance of three escapes granted two is in sample XI less than that of two escapes granted one, albeit the numbers are so small that the escape rates other than  $p_\alpha$  are poorly determined.

One could analyze in a similar manner the other samples in the monograph on measles in Providence; and if additional data were available for Providence or for other places so recorded as to make possible the tabulations that might be necessary, the analysis could be applied thereto. In



this way it would be possible to develop a detailed factual background on which some rational theory of the spread of measles in the family might in due time be built. Clearly the "family" might be a "schoolroom" or any other group of which a sufficient number of a specified type were available for study; but it may be long before sufficient numbers of groups other than families are available.

<sup>1</sup> Wilson, Edwin B., Bennett, Constance, Allen, Margaret, and Worcester, Jane, "Measles and Scarlet Fever in Providence, R. I., 1929-1934, with Respect to Age and Size of Family," *Proc. Amer. Philos. Soc., Philadelphia*, **80**, 357-476 (1939). See particularly pp. 441-453.

<sup>2</sup> Such a method has been used in economic statistics to discuss connectivity of fluctuations. See Wilson, Edwin B., "The Periodogram of American Business Activity," *Quarterly Journal of Economics*, May 1934, pp. 375-417, especially pp. 386, 409, 415.

<sup>3</sup> Yule, G. U., *Introduction to the Theory of Statistics*, or new edition by G. U. Yule and M. G. Kendall.

<sup>4</sup> In the symmetrical case where  $(A\beta) = (\alpha B)$  and  $p_A = p_B = s$ , the condition  $p_{AB}/s > p_A$  that "the chance of  $B$  if  $A$ " exceeds "the chance of  $A$ " is equivalent to the statement that the 4-fold table be positively associated.

<sup>5</sup> One may use as notation subscripts—those preceding the dot designating the group of which the probability is specified, those following the dot designating the attributes considered as granted.

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## HYBRIDIZATION BETWEEN *RANA PIPIENS* FROM VERMONT AND EASTERN MEXICO\*

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Communicated March 14, 1947

Cross fertilization experiments have been carried out among a number of geographic populations of *Rana pipiens* Schreber.<sup>1</sup> The intraspecific hybrids are normal if the distance between the localities from which the parents are obtained is relatively small. As the north-south, but not east-west, distance between the parents' places of origin is increased the hybrids exhibit progressively more severe defects in development. The most extreme cases previously observed involve crosses between Wisconsin (or Vermont) and Texas parents. If the cross is between the southern female and a northern male, the embryos are retarded in development and exhibit a considerable reduction of head structures. In the reciprocal cross, northern female and southern male, retardation in rate is observed, the circulatory system is defective, and head structures are greatly overdeveloped. The viability in both cases is low.

In order to ascertain if this gradient in hybrid inviability continues to

increase, crosses have been made between frogs from Mexico and Vermont. Material from two Mexican localities was available. One of these was Monterrey in Nuevo Leon and the other was the Rio Axtla, near the village of Axtla, in San Luis Potosi. These localities are 450 and 750 miles, respectively, south of the locality in Texas from which my previous material was obtained. All of the experiments which will be reported were conducted at 19.1°C.

*Experiment 1: Vermont ♀ × Axtla ♂.*—The types of defects previously described<sup>1</sup> for hybrids derived from northern males and southern females were observed. The yolk plug was abnormally large, the neural plate was very long, the distance between the neural folds in the head region was wider than normal, and development was retarded in post-gastrula stages. Cytolysis began in the neural fold stage and by the time the controls were in Stage 14L,<sup>2</sup> 31 per cent had died. Most of the embryos did not develop beyond Stages 16–17. Those which were able to continue development had extremely large heads and suffered circulatory system defects. One abnormal embryo, from a total of 155, reached the feeding stage before it died.

*Experiment 2: Vermont ♀ × Axtla ♂.*—These hybrids were more normal than those of Experiment 1. There was considerable retardation in rate of development but most of the embryos reached Stage 20. These had gigantic heads. The mucous glands were very large and secreted a thick brown mucous which hung from them in curtains. This was interpreted as an indication of hyper-activity of the mucous glands. The mouth was frequently plugged with endodermal cells. Seventy-seven per cent survived for 10 days; 2 per cent survived for 21 days; none survived as long as 29 days.

*Experiment 3: Vermont ♀ × Axtla ♂.*—All of these hybrids exhibited some degree of cytolysis and a few died in Stages 14–16. Thirty-four per cent survived to 104 hours, a time when the controls were in Stage 20. These had greatly enlarged heads. Nine per cent had a beating heart but none had gill circulation. One embryo, from a total of 188, survived to the twelfth day. It died shortly thereafter.

*Experiment 4: Vermont ♀ × Axtla ♂.*—These embryos exhibited marked cytolysis as neurulae. All but two, from a total of 247, died as gastrulae or neurulae. One of these two died on the fifth day and the other on the twelfth day.

*Experiment 5: Vermont ♀ × Monterrey ♂.*—Some of the embryos cytolized as neurulae but most formed abnormal embryos with large heads and circulatory system defects. Mucous secretion was abundant. All of the embryos were dead by the eleventh day.

*Experiment 6: Vermont ♀ × Monterrey ♂.*—In the neural fold stages 65 per cent showed extensive cytolysis. Cellular debris was coming off

from the neural groove. At this time 40 per cent had a perforation in the ventral epidermis which exposed the underlying germ layers. Seventeen per cent survived to the seventh day but they were very abnormal. All were dead by the fifteenth day.

*Experiment 7: Vermont ♀ × Monterrey ♂.*—This experiment gave the best hybrids secured in crosses of Vermont and Mexican individuals. There was little cytolysis in early stages but the usual type of morphological defect as well as retardation in rate of development was observed in the later stages. Twenty-two per cent survived to the sixteenth day and ten per cent are still alive on the one hundred and thirty-first day. Some of these will probably transform.

*Experiment 8: Vermont ♀ × Monterrey ♂.*—The male used in this cross was the same as in Experiment 7. In contrast with the previous case, the embryos of this experiment were the most abnormal hybrids in the entire series of crosses. The majority formed exogastrulae and none developed beyond the gastrula stage.

*Experiment 9: Axtla ♀ × Vermont ♂.*—The typical defects previously described<sup>1</sup> in hybrids derived from southern females and northern males were observed. The yolk plug was very small, the neural plate was short, the head and head structures were greatly reduced, and development was retarded in post-gastrula stages. Hatching was delayed long beyond the morphological stage in which it would normally occur. Some of the hybrids apparently died because they were unable to escape from their jelly membranes. Retardation in rate of development to Stage 20 was estimated as 66 per cent (in three experiments involving Texas females and either Wisconsin or Vermont males the retardation was 11, 11 and 8 per cent). Thirteen per cent of the embryos survived to the fourteenth day. Most of these died shortly thereafter except for one (1 per cent) which is still alive on the one hundred and fifty-eighth day. It is an albino.

*Experiment 10: Axtla ♀ × Vermont ♂.*—The same female was used as in Experiment 9 and development was essentially the same in the two cases. Sixteen per cent survived to the fourteenth day. A single embryo (1 per cent) is still alive on the one hundred and fifty-eighth day. It is an albino.

The defects observed in the Axtla ♀ × Vermont ♂ hybrids are essentially the same as those observed in Texas ♀ × Vermont ♂ crosses. Although retardation of development is greater in the Axtla female hybrids than in the Texas female hybrids, the morphological defects seem somewhat more extensive in the latter.

The cross Vermont ♀ × Mexico ♂ has produced the most extreme abnormalities so far observed in *Rana pipiens* racial crosses. The frequency of exogastrulation and the beginning of cytolysis as early in development as the gastrula and neurula stages is noteworthy. This is the basis for the

conclusion that the gradient in intraspecific hybrid inviability increases in Mexican populations of *Rana pipiens*.

In these experiments with intraspecific hybrids, we have reached a point where the extreme geographically separated populations are behaving towards one another as two "good" species. The defects observed in the hybrids are so extensive that the possibility of gene exchange between the two parent populations is most unlikely. At the same time adjacent populations appear to be perfectly interfertile. This paradox has been discussed previously and the conclusion reached that natural selection is promoting the development of different temperature-adapted races. The defects observed in the hybrids between northern and southern individuals are interpreted as a result of incompatibility in the interaction of a "low temperature genome" and a "high temperature genome" in the same zygote. The fact that the peripheral members are reproductively isolated from one another is merely a feature incidental to their adaptation.

It seems unlikely that isolating mechanisms such as sexual isolation, hybrid inviability and hybrid sterility are ever the *initial* isolating mechanisms in dividing a panmictic population of animals which reproduce sexually. Initial reproductive isolating mechanisms could best arise when the different parts of the population are physically separated through geographical, habitat or seasonal isolation. Under these conditions natural selection would be increasing the frequency of the genetic changes, which will subsequently act as isolating mechanisms, not for any value as isolating mechanisms but because they have some adaptive value in their special environment. A subsequent breakdown in effectiveness of geographical, habitat or seasonal isolation would bring two differently adapted populations into competition. If these produce hybrids which are inferior to the parental forms, then natural selection would favor the perfection of any differences which could serve as isolating mechanisms. In this manner the two "adaptive peaks" would remain distinct, and in fact be two species.

\* Aided by a grant from the Penrose Fund of the American Philosophical Society.

<sup>1</sup> Moore, J. A., *Genetics*, **31**, 304-326 (1946).

<sup>2</sup> These stages of development are described in Pollister, A. W., and Moore, J. A., *Anat. Rec.*, **68**, 489-496 (1937).

## A UNIQUENESS THEOREM FOR EIGENFUNCTION EXPANSIONS

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Let  $w_n(x, y)$  be the complete normal orthogonal eigenfunctions of the boundary value problem

$$\frac{\partial^2 w}{\partial x^2} + \frac{\partial^2 w}{\partial y^2} + \mu w = 0 \quad (1)$$

$$w(x, y) = 0 \text{ on } C. \quad (2)$$

Where  $C$  is the boundary of a regular region  $R$ , the eigenvalues  $\mu_n$  being arranged in non-decreasing order of magnitude. If  $f(x, y)$  be an arbitrary summable function defined in  $R$ , we may write

$$f(x, y) \sim \sum a_n w_n(x, y) \quad (3)$$

$$a_n = \int_D \int f w_n dx dy \quad (4)$$

the series on the right of (3) being called the *Fourier Eigenfunction Series* and  $a_n$  the *Fourier Coefficients* of  $f(x, y)$ . I have studied elsewhere<sup>1</sup> the problem of convergence and summability of a Fourier Eigenfunction Series. In this note I am interested in announcing a result on uniqueness of eigenfunction expansion. Actually, we have the following,

**THEOREM.** *Let us suppose we are given an eigenfunction series*

$$a_1 w_1(x, y) + a_2 w_2(x, y) + a_3 w_3(x, y) + \dots, a_1, a_2, \dots \text{ real} \quad (5)$$

*satisfying the following properties*

(i) *There exists a continuous function  $\phi(x, y)$  defined on  $D + C$  and vanishing on  $C$  such that*

$$\phi(x, y) \sim \sum \frac{a_n}{\mu_n} w_n(x, y) \quad (6)$$

(ii) *At every point in the region the series (5) is summable  $(J_1^2, \lambda_n)^2$  to a bounded measurable function  $f(x, y)$ , i.e.,*

$$\lim_{t \rightarrow 0} 4 \sum a_n w_n \frac{J_1^2(\lambda_n t)}{(\lambda_n t)^2} = f(x, y), \quad \lambda_n = (\mu_n)^{1/2} \quad (7)$$

*the series on the left converging for  $t > 0$ .*

*Then (5) is the Fourier eigenfunction series of  $f(x, y)$ .*

We indicate the general outlines of the proof:

The sum of the convergent series on the left of (7) defines, for every  $t > 0$ , a linear transformation of  $\phi$ :

$$4 \sum a_n w_n \frac{J_1^2(\lambda_n t)}{(\lambda_n t)^2} = U_t(\phi) \quad \text{say} \quad (8)$$

Then we have the following lemmas:

LEMMA 1. If for every  $(x, y)$  in  $D$ ,  $\lim_{t \rightarrow 0} U_t(\phi) \geq 0$  then  $\phi$  is subharmonic.

Similarly if  $\lim_{t \rightarrow 0} U_t(\phi) \leq 0$  then  $\phi$  is superharmonic.

So if  $U_t(\phi) \rightarrow 0$  as  $t \rightarrow 0$  then  $\phi$  is harmonic.

LEMMA 2.  $\lim U_t[\phi] \geq c$  for every  $(x, y)$  in  $R$  implies  $U_t(\phi) \geq c$  for some region contained in  $R$  with a similar conclusion if  $\lim U_t(\phi) \leq c$ .

From these lemmas we deduce that if  $|f(x, y)| \leq M$ , hypotheses (i) and (ii) of the theorem imply

$$|U_t(\phi)| \leq M.$$

Therefore

$$4a_n \frac{J_1^2(\lambda_n t)}{(\lambda_n t)^2} = \int_R U_t(\phi) w_n dx dy$$

and letting  $t \rightarrow 0$  we obtain the theorem.

We might add in conclusion, hypothesis (i) will be fulfilled if  $a_n$  tends to zero as rapidly as  $\mu_n^{-\epsilon}$  or  $(\log \mu_n)^{-1-\epsilon}$  or  $(\log \mu_n)^{-1} (\log \log \mu_n)^{-1-\epsilon}$ , etc. Thus if  $a_n$  satisfies any one of these conditions and  $\sum a_n w_n(x, y)$  converges everywhere to zero then  $a_n = 0$ ;  $n = 1, 2, \dots$

In the case of a double trigonometric series  $\sum_{\mu, \nu} C_{\mu, \nu} \exp i(\mu x + \nu y)$   $C_{00} = 0$  the theorem takes the following form:

(i)  $\sum \frac{C_{\mu, \nu}}{\mu^2 + \nu^2} \exp i(\mu x + \nu y)$  is the F.S. of a continuous periodic function  $\phi(x, y)$ .

$$(ii) \lim_{t \rightarrow 0} 4 \sum_1^\infty \frac{J_1^2(t\sqrt{n})}{nt^2} \sum_{\mu^2 + \nu^2 = n} \frac{C_{\mu, \nu}}{\mu^2 + \nu^2} \exp i(\mu x + \nu y) = f(x, y)$$

at every point  $(x, y)$  where  $f(x, y)$ , is a bounded measurable function. Then the trigonometric series is the F.S. of  $f(x, y)$ .

<sup>1</sup> Cf. "Notes on Fourier Expansions (1)," *Jour. Lond. Math. Soc.*, 20, 148-153 (1945) and the references therein.

<sup>2</sup> For definition of Bessel Summability, cf. the author's paper, "A New Summation Process," *Math. Student.*, 2, 21-27 (1943).



# ON THE CHARACTERISTIC CLASSES OF RIEMANNIAN MANIFOLDS

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The following imbedding theorem of sphere bundles was first stated, together with a sketched proof, by H. Whitney:<sup>1</sup> To a given bundle of spheres of dimension  $n - 1$  over a compact manifold  $M$  there exists a mapping  $f(M) \subset H(n, N)$  (which is the Grassmann manifold of all oriented linear spaces of dimension  $n$  through the origin of a real oriented Euclidean space  $E^{n+N}$  of  $n + N$  dimensions), which defines a sphere bundle over  $M$  equivalent to the given one, provided that  $\dim M \leq N$ . Steenrod<sup>2</sup> proved that if  $\dim M \leq N - 1$ , then two sphere bundles over  $M$  defined by the mappings  $f_i(M) \subset H(n, N)$ ,  $i = 1, 2$ , are equivalent, when and only when the mappings  $f_1$  and  $f_2$  are homotopic. From these theorems it would follow immediately that the inverse homomorphism  $f^*$  of the cohomology ring of  $H(n, N)$  into the cohomology ring of  $M$  is independent of the mapping  $f$  in the process of imbedding. But it was Pontrjagin<sup>3</sup> who first explicitly made this observation for the case of tangent sphere bundles and defined to be a characteristic cohomology class of  $M$  the image of a cohomology class of  $H(n, N)$  under the inverse homomorphism  $f^*$ . These characteristic cohomology classes include the ones which were first studied by Stiefel<sup>4</sup> and Whitney,<sup>5</sup> but they give many new ones.

Pontrjagin was interested mainly in tangent sphere bundles. He proved the imbedding theorem of tangent sphere bundles by simply imbedding the manifold  $M$  into  $E^{n+N}$  according to Whitney's imbedding theorem of differentiable manifolds. By being so imbedded,  $M$  acquires an induced Riemannian metric. In a second note<sup>6</sup> Pontrjagin showed that certain characteristic classes of  $M$  are expressible, in the sense of de Rham, by exterior differential forms constructed from the induced Riemannian metric. It is to be observed here that these characteristic classes are the images under  $f^*$  of the classes of  $H(n, N)$  which form a basis of the cohomology ring of  $H(n, N)$  with rational coefficients. This result of Pontrjagin includes, for instance, the now well-known generalization of the formula of Gauss-Bonnet for the case of an imbedded Riemannian manifold, as was first proved by Allendoerfer<sup>7</sup> and Fenchel.<sup>8</sup>

It is, however, not known whether the same results between the characteristic classes of  $M$  and the exterior differential forms of the Riemannian metric of  $M$  remain true, if the Riemannian metric is defined intrinsically on  $M$ . This question is significant, because the Riemannian metric of a differentiable manifold is in general not defined by imbedding the manifold

in a Euclidean space and it is not known whether an intrinsic Riemannian metric can be defined as an induced metric (in a Euclidean space), when the whole manifold is under consideration. It is the aim of this note to sketch a proof of the theorem that Pontrjagin's results remain true when the Riemannian metric on  $M$  is an intrinsic one. Complete details, together with generalizations to non-compact manifolds and affinely connected manifolds, will be published elsewhere.

We begin by stating the results of Pontrjagin in question. Let  $\Omega_k(i, k = 1, \dots, n)$  be the curvature forms of the Riemannian metric of  $M$ , in the notation of Elie Cartan. By exterior multiplication we construct from them the differential forms

$$\Delta_{4m} = (1/c_{4m}) \sum \Omega_{i_1 i_2} \Omega_{i_2 i_3} \dots \Omega_{i_{2m} i_1}, \quad 1 \leq m \leq n/4,$$

$$\Delta_0 = \sum \epsilon_{i_1 \dots i_n} \Omega_{i_1 i_2} \dots \Omega_{i_{n-1} i_n}, \quad \text{if } n \text{ is even,}$$

where  $\epsilon_{i_1 \dots i_n}$  is the Kronecker index and where the summations are extended over all the indices  $i$  from 1 to  $n$ . The number  $c_{4m}$  is a constant which is so chosen as to make the cocycle  $\Delta_{4m}$  an integral cocycle. In fact,  $c_{4m}$  is equal to  $m!$  times the total measure of all the straight lines in a spherical space of  $m + 1$  dimensions. The theorem, which Pontrjagin proved for the case of an induced Riemannian metric and which we shall prove to be true in general, is the following:

**THEOREM.** *Let  $M$  be a compact orientable Riemannian manifold. The differential forms  $\Delta_0, \Delta_{4m}$  ( $1 \leq m \leq n/4$ ) define cohomology classes which are characteristic cohomology classes of  $M$ .*

To prove this theorem we shall first give a description of the cohomology classes of  $H(n, N)$ . We take a sequence of integers  $a_1, \dots, a_n$  such that

$$a_1 \geq a_2 \geq \dots \geq a_n \geq 0$$

and a sequence of linear spaces

$$L_1 \subset L_2 \subset \dots \subset L_n$$

through the origin 0 of  $E^{n+N}$ , whose dimensions are

$$\dim L_i = N + i - a_i, \quad i = 1, \dots, n.$$

In  $H(n, N)$  we consider the linear spaces  $X$  such that

$$\dim (X \cap L_i) \geq i, \quad 1 \leq i \leq n.$$

The totality of these linear spaces, minus the ones on the boundary, forms a cell of dimension  $nN - \sum_{i=1}^n a_i$ . In particular, when the  $a$ 's satisfy certain conditions<sup>9</sup> which amount to say that certain integers constructed from them are even, the cell defines a cycle of dimension  $nN - \sum_{i=1}^n a_i$  and hence a

cocycle of dimension  $\sum_{i=1}^n a_i$  on  $H(n, N)$ , since  $H(n, N)$  is orientable. The corresponding cohomology class we shall denote by the symbol  $[a_1 \dots a_n]$ . Of particular importance for our purpose will be the cohomology classes  $\Gamma_{4m} = [2 \dots 2 \ 0 \dots 0]$  of dimension  $4m$ , where the symbol contains  $2m$  2's followed by  $n - 2m$  0's,  $m = 1, \dots, \left[ \frac{n}{4} \right]$ .

With these preliminaries we shall give the main steps of the proof in a number of lemmas.

LEMMA 1. *Over the base manifold  $M$  let  $\mathfrak{F}$  be the fibre space of the ordered sets of  $n - 2m + 2$  vectors satisfying the condition that they do not belong to a linear subspace of dimension  $n - 2m$ . If  $F$  denotes a fibre of  $\mathfrak{F}$ , then the  $i$ -dimensional homotopy groups  $\pi_i(F)$  of  $F$ ,  $0 < i \leq 4m - 2$ , vanish, while the  $(4m - 1)$ -dimensional homotopy group  $\pi_{4m-1}(F)$  is free cyclic. The cohomology class  $\Gamma_{4m}$  is the "obstacle cohomology class" (called by Steenrod<sup>10</sup> the characteristic class) of  $\mathfrak{F}$  in  $M$ .*

In fact, by a proper extension of the covering homotopy theorem to fibre spaces with exceptional fibres, it is not hard to show that  $F$  is simply connected. By a theorem of Hurewicz<sup>11</sup> the statements about the other homotopy groups reduce to corresponding statements about homology groups of the same dimension. By a retraction we can suppose that the sum of squares of all the components of the  $n - 2m + 2$  vectors is equal to one, so that  $F$  becomes a subspace of a sphere of  $n(n - 2m + 2) - 1$  dimensions. It then follows from Alexander's duality theorem that the homology groups  $H^i(F) = 0$ ,  $0 < i \leq 4m - 2$ , and that  $H^{4m-1}(F)$  is free cyclic. A generator of  $H^{4m-1}(F)$  can be given, by the explicit calculation of a linking coefficient. The last statement of the lemma follows almost immediately from a comparison of the definitions of the two cohomology classes.

LEMMA 2. *In the fibre bundle of all rectangular frames over  $M$  the differential form  $\Delta_{4m}$  is equal to the exterior derivative of a differential form of degree  $4m - 1$ , which reduces on a fibre to the form*

$$\frac{1}{e_{4m}} \sum \omega_{i_1 i_2} \dots \omega_{i_{4m-1} i_{4m}}$$

where  $e_{4m} \neq 0$  is a constant and where  $\omega_{ij}$  are the infinitesimal components of the frames.

The proof of this lemma is purely algebraic. It follows from certain identities for the exterior derivatives of some combinations of the differential forms. A particularly important rôle is played by these combinations which are formed from  $w_{ij}$ ,  $\Omega_{ij}$ , by a "cyclic summation" of the indices.

LEMMA 3. *In the group manifold  $O(n)$  of the proper orthogonal group in  $n$*

variables let  $O(n - 2m + 2)$  be a subgroup imbedded in  $O(n)$  in a definite way (for instance, by keeping the last  $2m - 2$  variables invariant). If  $4m \leq n$ , every cycle of dimension  $4m - 1$  of  $O(n)$  is homologous to a cycle of  $O(n - 2m + 2)$ .

This lemma follows from well-known results of Pontrjagin.<sup>12</sup>

Our theorem will be proved if we can identify  $\Gamma_{4m}$  with  $\Delta_{4m}$ . For this purpose we take a differentiable simplicial cycle  $z$  of dimension  $4m$  and show that its Kronecker product with  $\Gamma_{4m}$  is equal to the integral of  $\Delta_{4m}$  over it.  $z$  can be supposed to be in such a general position that there is a continuous field of  $n - 2m + 2$  vectors defined over  $z$  with only isolated points (to be called singular points) at which these vectors belong to a linear space of dimension  $n - 2m$ . The locus in  $M$  at which the  $n - 2m + 2$  vectors are linearly dependent is in general of dimension  $n - 2m + 1$  and its intersection with  $z$  of dimension  $2m + 1$ . It is therefore possible to suppose the vector field so chosen that the points at which the  $n - 2m + 2$  vectors are linearly dependent belong to the  $(4m - 1)$ -dimensional skeleton of  $z$ . Under these assumptions which do not affect the proof, we can deform the vector field into a field such that the  $n - 2m + 2$  vectors are mutually perpendicular unit vectors at each interior point of a  $4m$ -dimensional simplex of  $z$ , with the possible exception of one singular point.

In each (open) simplex of  $z$  there is at the same time defined a continuous field of  $(2m - 2)$ -dimensional planes with the possible exception of one singular point, namely the field of planes perpendicular to the  $n - 2m + 2$  vectors defined above. It is then possible to define a continuous field of ordered sets of  $2m - 2$  mutually perpendicular unit vectors which lie in these planes and which are such that they give rise to no new singularity.

To each simplex  $\sigma_i$  of  $z$  we construct a differentiable function (a "density")  $\lambda_i$ ,  $0 \leq \lambda_i \leq 1$ , such that  $\lambda_i = 0$  on the boundary of  $\sigma_i$  and that the content of the points of  $\sigma_i$  at which  $\lambda_i \neq 1$  approaches zero, where content is understood in the sense of Whitney. We define  $\lambda_i = 0$  for all points  $p \in z$ , which do not belong to  $\sigma_i$ . Then the integral  $\int_z \Delta_{4m}$  will differ as small as we please from the integral

$$\int_z (\sum_i \lambda_i) \Delta_{4m} = \sum_i \int_{\sigma_i} \lambda_i \Delta_{4m}.$$

Now in  $\sigma_i$  we have defined a continuous field of rectangular frames with a possible singularity at an interior point. We consider over  $\sigma_i$  the fibre bundle  $F^*$  of frames and integrate  $\lambda_i \Delta_{4m}$  in  $F^*$ . From Lemma 2 it is seen that  $\lambda_i \Delta_{4m}$  can be written as the exterior derivative of a differential form of degree  $4m - 1$ . The value of this integral is therefore contributed by the singularity and it follows from Lemma 3 that it is exactly the value assigned to  $\sigma_i$  by the cocycle  $\Gamma_{4m}$ . In this way we prove that  $\int_z \Delta_{4m}$  is equal to the Kronecker product of  $\Gamma_{4m}$  with  $z$ .

In conclusion we observe that it is not essential to assume our manifold to be compact, provided that the integration is carried out only over finite cycles. We also observe that similar results hold for affinely connected manifolds.<sup>13</sup>

<sup>1</sup> Whitney, H., *Bull. Amer. Math. Soc.*, **43**, 785-805 (1937).

<sup>2</sup> Steenrod, N., *Annals Math.*, **45**, 294-311 (1944).

<sup>3</sup> Pontrjagin, L., *C. R. (Doklady) Acad. Sci. USSR*, N. S., **35**, 34-37 (1942).

<sup>4</sup> Stiefel, E., *Comment. Math. Helv.*, **8**, 305-343 (1936).

<sup>5</sup> Whitney, *loc. cit.*

<sup>6</sup> Pontrjagin, L., *C. R. (Doklady) Acad. Sci. USSR*, N. S., **43**, 91-94 (1944).

<sup>7</sup> Allendoerfer, C. B., *Amer. J. Math.*, **62**, 243-248 (1940).

<sup>8</sup> Fenchel, W., *J. London Math. Soc.*, **15**, 15-22 (1940).

<sup>9</sup> See Pontrjagin, *loc. cit.*

<sup>10</sup> Steenrod, N., *Annals Math.*, **43**, 116-131 (1942).

<sup>11</sup> Hurewicz, W., *Proc. Acad. Sci. Amsterdam*, **38**, 521-528 (1935).

<sup>12</sup> Pontrjagin, L., *C. R. Acad. Sci. Paris*, **200**, 1277-1280 (1935).

<sup>13</sup> A preliminary note, entitled "Note on affinely connected manifolds," is due to appear in *Bull. Amer. Math. Soc.*

## ON THE FUNCTIONAL EQUATION $\frac{\partial}{\partial z} F(z, \alpha) = F(z, \alpha + 1)$

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We shall study differential-difference equations of the type

$$\frac{\partial}{\partial x} f(x, \alpha) = A(x, \alpha)f(x, \alpha) + B(x, \alpha)f(x, \alpha + 1), \quad (1)$$

where  $A(x, \alpha)$  and  $B(x, \alpha)$  are given functions. Included in equations of type (1) are the differential recurrence relations satisfied by Bessel, Legendre, Laguerre and Hermite functions. We shall motivate, discover and coördinate many of the formal properties of these functions by showing that they are special cases of a few simple formulae satisfied by certain classes of solutions of equations of the type (1). In this note we state the principal results only; proofs, additional theorems and examples, and explanations will appear in "An Essay on the Functional Equation  $\frac{\partial}{\partial z} F(z, \alpha) = F(z, \alpha + 1)$ ," forthcoming in the *Annals of Mathematics Studies*.

If  $f(x, \alpha)$  is a solution of the equation (1), and if

$$f(x, \alpha) \equiv \exp \left\{ - \int_{x_0}^x A(v, \alpha) dv \right\} f(x, \alpha) \quad (2)$$

then  $f(x, \alpha)$  satisfies the equation

$$\frac{\partial}{\partial x} f(x, \alpha) = B(x, \alpha) \exp \left\{ \int_{x_0}^x \Delta A(v, \alpha) dv \right\} f(x, \alpha + 1). \quad (3)$$

Hence we lose no generality in studying equations of the type

$$\frac{\partial}{\partial x} f(x, \alpha) = C(x, \alpha) f(x, \alpha + 1), \quad (4)$$

where  $C(x, \alpha)$  is a given function. We consider only the case when  $C(x, \alpha)$  is factorable:

$$C(x, \alpha) = X(x)A(\alpha). \quad (5)$$

If we make the change of variables

$$z = \int_{x_0}^x X(v) dv, \quad (6)$$

$$F(z, \alpha) = \exp \left\{ \sum_{\alpha_0}^{\alpha} \log A(v) \Delta v \right\} f(x, \alpha) \quad (7)$$

then we find that

$$\frac{\partial}{\partial z} F(z, \alpha) = F(z, \alpha + 1). \quad (8)$$

The equation (8), which we shall call "the  $F$ -equation," is the subject of our study.

**THEOREM 1.** *The factorability condition (5) is equivalent to the following condition on the coefficients of the original equation (1):*

$$\frac{\partial}{\partial x} \log B(x, \alpha) + \Delta A(x, \alpha) = \chi(x), \quad (9)$$

where  $\chi(x)$  is a function of  $x$  only.

The condition (9) is fulfilled by the coefficients of nearly all the differential-difference equations of type (1) satisfied by familiar special functions.

**THEOREM 2.** *Suppose  $f(x, \alpha)$  satisfies an equation of the type (1). If by transformations of the type*

$$F(z, \alpha) \equiv M(x, \alpha) f(x, \alpha), \quad z \equiv \phi(x), \quad (10)$$

*there may be derived from  $f(x, \alpha)$  two different functions  $F_1(z, \alpha)$  and  $F_2(z, \alpha)$  both satisfying the  $F$ -equation, then there exist constants  $k$  and  $l$  and a periodic function  $\pi(\alpha)$  of period 1 such that*

$$F_2(z, \alpha) = \pi(\alpha) k^\alpha F_1(kz + l, \alpha). \quad (11)$$

*Solutions of the  $F$ -Equation Involving Familiar Functions.*—In the follow-



ing list  $z$  and  $\alpha$  are the (complex) variables of the  $F$ -equation, and any other parameters  $\gamma$ ,  $\delta$ , etc., are constants

EXPRESSION	NAME OF FUNCTION INVOLVED
1. $e^{i\alpha\pi} z^{-\alpha/2} Z_{\alpha}(2\sqrt{z})$ } 2. $z^{-\alpha/2} Z_{-\alpha}(2\sqrt{z})$ }	Bessel or Hankel function of either kind
3. $e^{i\alpha\pi} e^{-z} L_{\gamma}^{(\alpha)}(z)$ 4. $z^{-\alpha} L_{\gamma}^{(-\alpha)}(z)/\Gamma(\gamma - \alpha + 1)$	
5. $\Gamma(\alpha + 1)(-z)^{-\alpha-1-\gamma} e^{-1/z} L_{\alpha}^{(\gamma)}\left(\frac{1}{z}\right)$ 6. $\Gamma(\alpha - \gamma)(-z)^{-\alpha} L_{-\alpha}^{(\gamma)}\left(\frac{1}{z}\right)$	Laguerre function
7. $e^{-2\sqrt{z}} {}_1F_1(\alpha; 2\alpha; 4\sqrt{z})/\Gamma(\alpha + 1/2)$	
8. $e^{-z^2} H_{\alpha}(-z)$ } 9. $\Gamma(\alpha) H_{-\alpha}(-z/2)$ }	Hermite function
10. $\Gamma(\alpha - \beta + 1)(z^2 - 1)^{-\alpha+1/2} \mathfrak{P}_{\alpha}^{\beta}\left(-\frac{z}{\sqrt{z^2 - 1}}\right)$	
11. $\Gamma(\alpha - \beta)(z^2 - 1)^{-\alpha/2} \mathfrak{P}_{-\alpha}^{\beta}\left(-\frac{z}{\sqrt{z^2 - 1}}\right)$	Associated Legendre function of either kind
12. $(1 - z^2)^{-\alpha/2} \mathfrak{P}_{\beta}^{\alpha}(z)$	

There are at least 26 more solutions involving familiar special functions, some of these being functions occurring in the theory of numbers and in mathematical statistics.

We now list a number of theorems concerning solutions of the  $F$ -equation. In the examples following the theorems the numbers in parentheses refer to the above list of solutions, and indicate that the solution(s) of that(those) number(s) need only be substituted for  $F(z, \alpha)$  in the formula given by the theorem in order for the formula given as an example to follow immediately. Of these examples, formulae (15), (27), (33), (42), (43), (48), (49), (55) and (77) are to the best of my knowledge new results, while formulae (16), (32), (37), (38), (39), (52), (57), (58), (60), (61), (67), (69), (70) and (72) are generalizations of known results.

It is very easy to prove any one of these formulae, once one sees it set up. In the study of special functions the difficulty lies first in discovery and second in coördination of results. In no case in applying the theorems of this note do we need to know in advance the formula that will result in a particular example. We ask simply for a formula of a general type, e.g., an infinite exponential integral involving Laguerre functions, a contour integral for Bessel functions or a formula expressing Laguerre functions in terms of Bessel functions, and each time we find the resulting expression in a nearly automatic fashion. The purpose of all examples is to illustrate the

discovering and correlative value of the theorems. There are many more interesting examples than those given here.

Latin  $m, n, p, r$  represent positive integers only; Greek letters always represent complex quantities.

THEOREM 3. *If  $F(z, \alpha)$  satisfies the  $F$ -equation, then*

$$F(z, \alpha + n) = \frac{\partial^n}{\partial z^n} F(z, \alpha). \quad (12)$$

Examples 3a: (1, 2; Bessel)

$$(-)^n x^{-\alpha-n} J_{\alpha+n}(x) = \frac{d^n}{(x dx)^n} [x^{-\alpha} J_{\alpha}(x)]. \quad (13)$$

$$x^{\alpha-n} J_{\alpha-n}(x) = \frac{d^n}{(x dx)^n} [x^{\alpha} J_{\alpha}(x)]. \quad (14)$$

Example 3b: (5)

$$L_n^{(\gamma)}\left(\frac{1}{x}\right) = \frac{(-)^n}{n!} x^{1+\gamma} e^{1/x} \frac{d^n}{dx^n} [x^{-1-\gamma} e^{-1/x}]. \quad (15)$$

Examples 3c: (10; known special case of formula (16):  $\alpha = 0$ )

$$P_{\alpha+n}^{\alpha}(\cos \theta) = \frac{(-)^n \Gamma(2\alpha + 1)}{n! 2^{\alpha} \Gamma(\alpha + 1)} \csc^{\alpha+n+1} \theta \frac{d^n \sin^{2\alpha+1} \theta}{d(\cot \theta)^n} \quad (16)$$

$$Q_n(\cos \theta) = \frac{(-)^n}{n!} \csc^{n+1} \theta \frac{d^n}{d(\cot \theta)^n} \{\sin \theta \log(\cot \theta + \csc \theta)\}. \quad (17)$$

THEOREM 4. *If  $\phi(\alpha)$  be such that*

$$\lim_{n \rightarrow \infty} \frac{\phi(\alpha_0 + n + 1)}{n\phi(\alpha_0 + n)} = \frac{1}{k} \quad (18)$$

*then whenever  $|z - z_0| < k$  and  $\alpha = \alpha_0 + i, i = 0, 1, 2, \dots$ , there exists a unique solution  $F(z, \alpha)$  of the  $F$ -equation such that  $F(z_0, \alpha) = \phi(\alpha)$ . This solution may be represented by the power series*

$$F(z, \alpha) = \sum_{n=0}^{\infty} \phi(\alpha + n) \frac{(z - z_0)^n}{n!}. \quad (19)$$

Examples 4a: (3, 7; Kummer)

$$e^z {}_1F_1(-\gamma; \alpha; z) = F(\alpha + \gamma; \alpha; -z). \quad (20)$$

$$e^{-2\sqrt{z}} {}_1F_1(\alpha; 2\alpha; 4\sqrt{z}) = {}_0F_1(\alpha + 1/2; z). \quad (21)$$

THEOREM 5 (Doetsch). *In Theorem 4,  $F(z, \alpha)$  may be represented in the form*

$$F(z, \alpha) = e^{z-z_0} \sum_{n=0}^{\infty} \frac{(z-z_0)^n}{n!} \Delta^n \phi(\alpha). \quad (22)$$

COROLLARY (Euler). *If the series on the left is convergent,*

$$e^{-x} \sum_{n=0}^{\infty} a_n \frac{x^n}{n!} = \sum_{n=0}^{\infty} \Delta^n a_0 \frac{x^n}{n!}. \quad (23)$$

THEOREM 6. *If  $\phi(\alpha)$  is representable in a Newton series,*

$$\phi(\alpha) = \sum_{n=0}^{\infty} (-)^n a_n \binom{\alpha-1}{n}, \quad \Re \alpha \geq \alpha_0, \quad (24)$$

*then in Theorem 4  $F(z, \alpha)$  may also be represented by a Newton series:*

$$F(z, \alpha) = \sum_{n=0}^{\infty} (-)^n a_n (z-z_0) \binom{\alpha-1}{n}, \quad \Re \alpha \geq \alpha_0, \quad (25)$$

where

$$a_n(t) = \sum_{p=0}^{\infty} (-)^p a_{n+p} \frac{t^p}{p!}. \quad (26)$$

Example 6: (For  $\phi(\alpha)$  put  $F(1-\alpha, \beta; \gamma; \delta)$ , and rearrange.)

$$L_p^{(m+\epsilon)}(z) = (-)^m \sum_{n=0}^m (-)^n \binom{m}{n} L_{m+p}^{(n+\epsilon)}(z). \quad (27)$$

THEOREM 7. *If in Theorem 4  $\phi(\alpha)$  may be represented as a contour integral,*

$$\phi(\alpha) = \int_C \psi_t(\alpha) dt, \quad \Re \alpha \geq \alpha_0, \quad (28)$$

and if  $|\psi_t(\alpha)| < M$  when  $\Re \alpha \geq \alpha_0$ , then

$$F(z, \alpha) = \int_C \sum_{n=0}^{\infty} \psi_t(\alpha+n) \frac{(z-z_0)^n}{n!} dt, \quad \Re \alpha \geq \alpha_0. \quad (29)$$

Example 7a: (1; Schläfli)

$$J_\alpha(2\sqrt{z}) = \frac{z^{\alpha/2}}{2\pi i} \int_{-\infty}^{(0+)} t^{-\alpha-1} \exp\left(t - \frac{z}{t}\right) dt. \quad (30)$$

Example 7b: (3; Sonine)

$$L_\beta^{(\alpha)}(z) = \frac{\Gamma(\alpha+\beta+1)}{\Gamma(\beta+1)2\pi i} \int_{-\infty}^{(0+)} \left(1 - \frac{z}{t}\right)^\beta t^{-\alpha-1} e^t dt. \quad (31)$$

Example 7c: (1; case  $\beta = \alpha + 1/2$ ; Hankel)

$$J_{\alpha}(2\sqrt{z}) = \frac{-e^{-i\alpha\pi} z^{\beta/2}}{4i\Gamma(\beta) \sin \beta\pi \sin(\alpha - \beta)\pi} \int_A^{(1+, 0+, 1-, 0-)} t^{(\alpha-\beta)/2} (1-t)^{\beta-1} J_{\alpha-\beta}(2\sqrt{zt}) dt. \quad (32)$$

Example 7d: (3)

$$L_{\beta}^{(\alpha)}(z) = \frac{e^{-i\alpha\pi} \Gamma(\alpha + \beta + 1)}{4i\Gamma(\gamma) \Gamma(\alpha + \beta + 1 - \gamma) \sin \gamma\pi \sin(\alpha - \gamma)\pi} \int_A^{(1+, 0+, 1-, 0-)} t^{\alpha-\gamma} (1-t)^{\gamma-1} L_{\beta}^{(\alpha-\gamma)}(zt) dt. \quad (33)$$

THEOREM 8. If  $F(z, \alpha)$  is an analytic solution of the  $F$ -equation, then

$$F(z + h, \alpha) = \sum_{n=0}^{\infty} \frac{h^n}{n!} F(z, \alpha + n). \quad (34)$$

Examples 8a: (1, 2; Lommel)

$$(z + h)^{-\alpha/2} J_{\alpha}(2\sqrt{z + h}) = \sum_{n=0}^{\infty} \frac{(-h)^n}{n!} z^{-(\alpha+n)/2} J_{\alpha+n}(2\sqrt{z}). \quad (35)$$

$$(z + h)^{\alpha/2} J_{\alpha}(2\sqrt{z + h}) = \sum_{n=0}^{\infty} \frac{h^n}{n!} z^{(\alpha-n)/2} J_{\alpha-n}(2\sqrt{z}). \quad (36)$$

Examples 8b: (10; special cases of formula (37):  $\alpha = 0, \beta = 0$ , de Louville, Legendre;  $\alpha = \beta$ , Gegenbauer; known special case of formula (38):  $m = 0, t = 1/x$ ; special case of formula (39):  $\alpha = 0, \beta = 0$ , Didon)

$$(t^2 - 2tx + 1)^{-(\alpha+1)/2} P_{\alpha}^{\beta}\left(\frac{x-t}{\sqrt{t^2 - 2tx + 1}}\right) = \sum_{n=0}^{\infty} \binom{\alpha - \beta + n}{n} t^n P_{\alpha+n}^{\beta}(x). \quad (37)$$

$$(t^2 - 2tx + 1)^{p/2} P_p^m\left(\frac{x-t}{\sqrt{t^2 - 2tx + 1}}\right) = \sum_{r=0}^{p-m} \binom{p+m}{r} (-)^r P_{p-r}^m(x). \quad (38)$$

$$(t^2 - 2tx + 1)^{-(\alpha+1)/2} Q_{\alpha}^{\beta}\left(\frac{x-t}{\sqrt{t^2 - 2tx + 1}}\right) = \sum_{n=0}^{\infty} \binom{\alpha - \beta + n}{n} t^n Q_{\alpha+n}^{\beta}(x) \quad (39)$$

Example 8c: (5; Sonine, Pinney)

$$(1-t)^{-\alpha-\gamma-1} \exp\left(-\frac{xt}{1-t}\right) L_{\alpha}^{(\gamma)}\left(\frac{x}{1-t}\right) = \sum_{n=0}^{\infty} \binom{\alpha+n}{n} t^n L_{\alpha+n}^{(\gamma)}(x). \quad (40)$$

THEOREM 9. If  $F(z, \alpha)$  is a solution of the  $F$ -equation, then

$$\sum_{n=0}^{\infty} F(z, \alpha + n) t^n = \int_0^{\infty} e^{-\theta} F(z + \theta t, \alpha) d\theta, \quad (41)$$

provided that both the series and the integral converge uniformly.

Example 9a: (8)

$$\frac{\sqrt{\pi}}{2t} \exp \left[ \left( \frac{1}{2t} - z \right)^2 \right] \operatorname{erfc} \left( \frac{1}{2t} - z \right) = \sum_{n=0}^{\infty} t^n H_n(z). \quad (42)$$

Example 9b: (1, and a result of Schlömilch)

$$\int_0^{\infty} e^{-\theta} \left\{ J_0 \sqrt{x^2 - 2\theta\tau x} + J_0 \sqrt{x^2 + \frac{2\theta x}{\tau}} \right\} d\theta = J_0(x) + \exp \left[ x \left( \tau - \frac{1}{\tau} \right) / 2 \right]. \quad (43)$$

THEOREM 10. If  $F(z, \alpha)$  is a solution of the  $F$ -equation such that, for fixed values of  $\alpha$  and  $z$ ,

$$\lim_{n \rightarrow \infty} \frac{F(z, \alpha - n - 1)}{F(z, \alpha - n)} = \frac{1}{k}, \quad k = k(z, \alpha), \quad (44)$$

and if

A.  $F(z_1, \alpha) = 0$ ,  $\alpha = \alpha_0 + i$ ,  $i = 0, 1, 2, \dots$ , and  $F(z, \alpha)$  is integrable over the range  $(0, z_1 - z)$ , then when  $|t| < k$

$$\sum_{n=0}^{\infty} F(z, \alpha - n) t^n = \int_{z_1}^z e^{(z-\xi)t} F(\xi, \alpha + 1) d\xi; \quad (45)$$

B.  $\int_0^{\infty} F(z + \theta, \alpha) d\theta$  exists, but  $F(z, \alpha + 1)$  does not vanish, then when  $|t| < k$

$$\sum_{n=0}^{\infty} F(z, \alpha - n) t^n = - \int_0^{\infty} e^{-\theta t} F(z + \theta, \alpha + 1) d\theta; \quad (46)$$

C. (Appell)  $F(z, \alpha + 1) \equiv 0$ , then when  $|t| < k$

$$\sum_{n=0}^{\infty} F(z, \alpha - n) t^n = e^{zt} \sum_{n=0}^{\infty} F(0, \alpha - n) t^n. \quad (47)$$

Examples 10a: (4; formula (50): Deruyts)

$$\sum_{n=0}^{\infty} \frac{z^{-\alpha+n} L_{\gamma}^{(-\alpha+n)}(z)}{\Gamma(-\alpha+n+\gamma+1)} t^n = \frac{1}{\Gamma(\gamma-\alpha)} \int_0^z e^{(z-\xi)t} \xi^{-\alpha-1} L_{\gamma}^{(-\alpha-1)}(\xi) d\xi, \quad \Re \alpha < 0. \quad (48)$$

$$\sum_{n=0}^{\infty} \frac{z^{-\alpha+n} L_p^{(-\alpha+n)}(z)}{\Gamma(-\alpha+n+p+1)} t^n = \frac{1}{\Gamma(p-\alpha)}$$

$$\int_0^\infty e^{-\theta t} (z + \theta)^{-\alpha-1} L_p^{(-\alpha-1)}(z + \theta) d\theta, \Re \alpha > p + 1, \alpha \text{ not an integer}; \quad (49)$$

$$\sum_{n=0}^{\infty} \frac{z^{-p+n} L_p^{(-p+n)}(z)}{n!} t^n = \frac{(t-1)^p}{p!} e^t. \quad (50)$$

Examples 10b: (6; Sonine)

$$\sum_{n=0}^{\infty} L_n^{(\gamma)}(x) \frac{t^n}{\Gamma(\gamma + n + 1)} = e^t (xt)^{-\alpha/2} J_\alpha(2\sqrt{xt}). \quad (51)$$

Example 10c: (11; case  $m = 0$ ; Catalan)

$$\sum_{n=0}^{\infty} \frac{t^n}{(m+n)!} P_{m+n}^m(x) = e^t (1-x^2)^{m/2} \frac{2^m [\Gamma(m+1/2)]^2}{\pi m!} {}_2F_3(m+1/2, m+1/2; 1/2, m/2+1/2, m/2+1; -\frac{t^2}{4}(1-x^2)). \quad (52)$$

THEOREM 11. Suppose that  $F(z, \alpha)$  is a solution of the  $F$ -equation such that

- (i)  $\int_0^c t^{\alpha+k} F(t, \alpha) dt$  exists and represents an analytic function of  $\alpha$  in some strip  $\alpha_0 \leq \Re \alpha \leq \alpha_0 + 1, \alpha_0 + \Re k > -1$ ;
- (ii)  $F(t, \alpha)$  is a continuous function of  $t$  when  $0 \leq t \leq c, \alpha_0 \leq \Re \alpha \leq +1$ ;
- (iii)  $F(c, \alpha) = 0, \alpha_0 \leq \Re \alpha \leq \alpha_0 + 1$ ;
- (iv)  $e^{-t\alpha\pi} \int_0^c t^{\alpha+k+1} F(t, \alpha) dt / \Gamma(\alpha + k + 1)$  is a bounded function of  $\alpha$  everywhere in the strip  $\alpha_0 \leq \Re \alpha \leq \alpha_0 + 1$ .

Then

$$\int_0^c t^{\alpha+k} F(t, \alpha) dt = e^{t\alpha\pi} \Gamma(\alpha + k + 1) f(k), (\Re \alpha + k) > -1. \quad (53)$$

Example 11a: (1; Lipschitz, Weber)

$$\int_0^\infty t^{\alpha/2+k} J_\alpha(2\sqrt{t}) dt = \frac{\Gamma(\alpha + k + 1)}{\Gamma(-k)}, \Re(-k - 1/2) > \Re \alpha > -1. \quad (54)$$

Example 11b: (8)

$$\int_0^\infty e^{-t^2} t^\alpha + H_\alpha^k(-t) dt = \frac{e^{t\alpha\pi} \Gamma(\alpha + k + 1) \Gamma(1/2)}{2^{k+1} \Gamma(k/2 + 1)}, \quad \Re(\alpha + k) > 1. \quad (55)$$

THEOREM 12. Let  $F(z, \alpha)$  be a solution of the  $F$ -equation such that  $F(z_0, \alpha) = \phi(\alpha)$ . Then when the integral and the series are both uniformly convergent,

$$\int_0^\infty e^{-(At)^{1/m}} t^{\alpha+\gamma-1} F((z-z_0)t + z_0, \alpha) dt = \frac{m}{A^{\alpha+\gamma}} \sum_{n=0}^{\infty} \frac{\Gamma(m\alpha + m\gamma + mn)}{n!} \phi(\alpha + n) \left( \frac{z-z_0}{A} \right)^n. \quad (56)$$

Example 12a: (1; cases  $m = 1, 2$ ; Lipschitz, Weber, Hankel, Hobson, and others)



$$\int_0^\infty e^{-(At)^{1/m}} t^{\alpha/2+\gamma-1} J_\alpha(2\sqrt{zt}) dt = \frac{mz^{\alpha/2}\Gamma(m\gamma+m\alpha)}{A^{\alpha+\gamma}\Gamma(\alpha+1)} {}_mF_1\left(\alpha+\gamma, \alpha+\gamma+\frac{1}{m}, \dots, \alpha+\gamma+\frac{m-1}{m}; \alpha+1; -\frac{zm^m}{A}\right). \quad (57)$$

Example 12b: (3; case  $m = 1, \delta = 1, z = 1$ : Sonine)

$$\int_0^\infty e^{-u-(At)^{1/m}} t^{\alpha+\delta-1} L_\gamma^{(\alpha)}(zt) dt = \frac{m\Gamma(m\alpha+m\delta)\Gamma(\alpha+\gamma+1)}{A^{\alpha+\delta}\Gamma(\alpha+1)\Gamma(\gamma+1)} {}_{m+1}F_1\left(\alpha+\delta, \alpha+\delta+\frac{1}{m}, \dots, \alpha+\delta+\frac{m-1}{m}, \alpha+\gamma+1; \alpha+1; -\frac{zm^m}{A}\right). \quad (58)$$

THEOREM 13. If  $F(z, \alpha)$  is a solution of the  $F$ -equation, then when both the series and the integral are uniformly convergent,

$$\int_0^1 t^{\alpha+\beta-1} (1-t^{1/m})^{m\gamma-1} F((z-z_0)t+z_0, \alpha) dt = m\Gamma(m\gamma) \sum_{n=0}^\infty \frac{\Gamma(m\alpha+m\beta+mn)}{\Gamma(m\alpha+m\beta+m\gamma+mn)} \phi(\alpha+n) \frac{(z-z_0)^n}{n!}. \quad (59)$$

Example 13a: (1; case  $m = 1, \beta = 1$ : Sonine)

$$\int_0^1 t^{\alpha/2+\beta-1} (1-t^{1/m})^{m\gamma-1} J_\alpha(2\sqrt{zt}) dt = \frac{mz^{\alpha/2}\Gamma(m\alpha+m\beta)\Gamma(m\gamma)}{\Gamma(m\alpha+m\beta+m\gamma)\Gamma(\alpha+1)} {}_mF_{m+1}\left(\alpha+\beta, \alpha+\beta+\frac{1}{m}, \dots, \alpha+\beta+\frac{m-1}{m}; \alpha+\beta+\gamma, \alpha+\beta+\gamma+\frac{1}{m}, \dots, \alpha+\beta+\gamma+\frac{m-1}{m}, \alpha+1; -z\right). \quad (60)$$

Example 13b: (3; case  $\delta = \alpha + 1$ : Koshliakoff)

$$\int_0^1 t^{\delta-1} (1-t)^{\gamma-1} L_\epsilon^{(\alpha)}(zt) dt = \frac{\Gamma(\gamma)\Gamma(\delta)\Gamma(\alpha+\epsilon+1)}{\Gamma(\alpha+1)\Gamma(\delta+1)\Gamma(\delta+\gamma)} {}_2F_2(-\epsilon, \delta; \alpha+1, \delta+\gamma; z). \quad (61)$$

THEOREM 14. Suppose  $F(z, \alpha)$  is a solution of the  $F$ -equation, and suppose the functions  $F_i(z, \alpha)$  form a set of solutions of the  $F$ -equation. Let  $O_t$  be an operator which binds the variable  $t$  and which commutes, in the formula (63) below, with the operations of differentiation with respect to  $z$ , replacing  $\alpha$  by  $\alpha + 1$ , and replacing  $z$  by  $z_0$ . Then if the relation

$$F(z_0, \alpha) = O_t[F_t(z_0, \alpha)], \quad \Re \alpha \geq \alpha_0, \quad (62)$$

holds for a fixed value  $z_0$  of  $z$ , it holds for all values of  $z$  such that it has a meaning:

$$F(z, \alpha) = O_t[F_1(z, \alpha)], \quad \Re \alpha \geq \alpha_0. \quad (63)$$

COROLLARY 14A. Let  $F_1(z, \alpha)$  and  $F_2(z, \alpha)$  be two solutions of the  $F$ -equation. Suppose that there exists an operator  $O_t$  as in Theorem 14 and a function  $G(t)$  such that

$$F_2(z_0, \alpha) = F_1(z_0, \alpha) O_t[\{G(t)\}^\alpha], \quad \Re \alpha \geq \alpha_0. \quad (64)$$

Then if  $O_t$  has the further property  $O_t[f(z, \alpha)g(t)] = f(z, \alpha)O_t[g(t)]$ , it follows that

$$F_2(z, \alpha) = O_t[\{G(t)\}^\alpha F_1((z - z_0)G(t) + z_0, \alpha)], \quad \Re \alpha \geq \alpha_0, \quad (65)$$

for all values of  $z$  for which the expression on the right has a meaning.

Example 14Aa: Problem: to find a formula expressing  $L_\gamma^{(\alpha)}(x)$  in terms of  $J_\alpha(x)$ . Consider solutions nos. 1 and 3. Substitute in formula (64) when  $z = 0$ . Then we need to find  $G(t)$  and  $O_t$  such that

$$\Gamma(\alpha + \gamma + 1)/\Gamma(\gamma + 1) = O_t[\{G(t)\}^\alpha].$$

A suitable selection is given by the formulae

$$G(t) \equiv t,$$

$$O_t[\dots] \equiv \frac{1}{\Gamma(\gamma + 1)} \int_0^\infty e^{-t\gamma} [\dots] dt.$$

Formula (65) then gives Hankel's integral

$$e^{-z\alpha/2} L_\gamma^{(\alpha)}(z) = \frac{1}{\Gamma(\gamma + 1)} \int_0^\infty e^{-t\gamma + \alpha/2} J_\alpha(2\sqrt{zt}) dt. \quad (66)$$

To invert this integral, interchange the rôles of solutions 1 and 3 in formula (64). We then find a generalization of formula (30):

$$z^{-\alpha/2} J_\alpha(2\sqrt{z}) = \frac{\Gamma(\gamma + 1)}{2\pi i} \int_{-\infty}^{(0+)} e^{-z/t} t^{-\alpha-\gamma-1} L_\gamma^{(\alpha)}\left(\frac{z}{t}\right) dt, \quad (67)$$

Example 14Ab: Problem: to find a formula expressing  $J_{\alpha+\beta}(x)$  in terms of  $J_\alpha(x)$ . We use the solution no. 1 for both  $F_1(z, \alpha)$  and  $F_2(z, \alpha)$  in Corollary 14A. Using the definitions

$$G(t) \equiv \sin^2 t$$

$$O_t[\dots] \equiv \frac{e^{i\beta\pi}}{2\Gamma(\beta)} \int_0^{\pi/2} \cos 2\theta^{-1} \sin t [\dots] dt,$$

we discover Sonine's formula

$$\left(\frac{x}{2}\right)^{-\beta} J_{\alpha+\beta}(x) = \frac{1}{2\Gamma(\beta)} \int_0^{\pi/2} J_{\alpha}(x \sin t) \sin^{2\alpha+1} t \cos^{2\beta-1} t dt. \quad (68)$$

*Example 14Ac:* Problem: to find a formula giving the Hermite functions in terms of the Legendre functions. We use solutions nos. 3 and 10 and the choices

$$G(t) \equiv \sqrt{t},$$

$$O_t[\dots] \equiv \frac{2^{\beta}}{\sqrt{\pi}} \int_0^{\infty} e^{-t} t^{-1/2+\beta/2} [\dots] dt.$$

We find then that

$$(1-x^2)^{\beta/2} P_{\alpha}^{\beta}(x) = \frac{2^{\beta+1}}{\sqrt{\pi}\Gamma(\alpha-\beta+1)} \int_0^{\infty} e^{-\theta^2} \theta^{\alpha+\beta} H_{\alpha-\beta}(x\theta) d\theta. \quad (69)$$

Similar methods enable us to find an inverse:

$$H_{\gamma}(z) = \frac{\Gamma(\gamma+1)}{2\beta\sqrt{\pi}i} \int_{+\infty}^{-\infty} e^{-\theta^2} (-\theta^2 - z^2)^{-\beta/2} (-\theta)^{-\beta-\gamma} P_{\beta+\gamma}^{\beta}\left(\frac{iz}{\theta}\right) d\theta. \quad (70)$$

Case  $\beta = 0$ : Glaisher, Curzon.

**COROLLARY 14B.** If  $F_1(z, \alpha)$  and  $F_2(z, \alpha)$  are solutions of the  $F$ -equation, and if for some value of  $z$  the relation

$$F_2(z, \alpha) = O_t[F_1(z+t, \alpha)], \quad \Re \alpha \geq \alpha_0, \quad (71)$$

is correct, then it is correct for all values of  $z$  for which the expression on the right has a meaning.

*Example 14B:* (4, and a special case of formula (58); case  $z = 0$ : Sonine)

$$\int_0^{\infty} e^{-yt} (t+z)^{\alpha} L_{\gamma}^{(\alpha)}(t+z) dt = \frac{\Gamma(\alpha+\gamma+1)(y-1)^{\gamma}}{\Gamma(\gamma+1)y^{\gamma+\alpha+1}} e^{zy}. \quad (72)$$

There are other interesting special cases of Theorem 14 not included in these two corollaries.

**THEOREM 15 (Doetsch).** Suppose  $F(z, \alpha)$  is any solution of the  $F$ -equation such that  $F(z_0, \alpha_0 - j)$ ,  $j = 0, 1, 2, \dots, m$ , exists. Then

$$F(z, \alpha_0 - m) = \int_{z_0}^z F(t, \alpha_0) \frac{(z-t)^{m-1}}{(m-1)!} dt + \sum_{j=1}^m F(z_0, \alpha_0 - j) \frac{(z-z_0)^{m-j}}{(m-j)!} \quad (73)$$

*Examples 15a:* (1, 2; Sonine)

$$z^{-(\alpha-m)/2} J_{\alpha-m}(2\sqrt{z}) = \frac{1}{\Gamma(m)} \int_0^\infty t^{m-1} (z+t)^{-\alpha/2} J_\alpha(2\sqrt{z+t}) dt. \quad (74)$$

$$z^{(\alpha+m)/2} J_{\alpha+m}(2\sqrt{z}) = \frac{1}{\Gamma(m)} \int_0^z t^{\alpha/2} (z-t)^{m-1} J_\alpha(2\sqrt{t}) dt. \quad (75)$$

*Example 15b:* (5; Koshliakoff)

$$z^{m+\alpha} L_\gamma^{(\alpha+m)}(z) = \frac{\Gamma(\alpha+m+\gamma+1)}{\Gamma(m)} \int_0^z t^\alpha (z-t)^{m-1} L_\gamma^{(\alpha)}(t) dt. \quad (76)$$

*Example 15c:* (10)

$$(x^2-1)^{\alpha/2} P_{\alpha-m}^\beta(x) = m \binom{\alpha-\beta}{m} \int_{-1}^x (\theta^2-1)^{(\alpha-m-1)/2} (\theta\sqrt{x^2-1} - x\sqrt{\theta^2-1})^{m-1} P_\alpha^\beta(\theta) d\theta. \quad (77)$$



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## STUDIES ON THE BIOCHEMISTRY OF *TETRAHYMENA*. IX. FOLIC ACID COMPONENTS AND CONJUGATES\*

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*Introduction.*—The recent publication of the reports of the conference on folic acid<sup>1</sup> has served to clear up many confusing points in the literature on this subject. It is possible now to substitute chemical terms for such names as "folic acid," "Vitamin B<sub>c</sub>," "*L. casei* factor," "norit eluate factor," "Vitamin M," etc., which are considered synonymous with pteroylglutamic acid; "fermentation folic acid," which is pteroyldiglutamyl glutamic acid; and "Vitamin B<sub>c</sub> conjugate," which is pteroylhexaglutamyl glutamic acid. These three members of the "folic acid group" possess widely different activities for the lactic acid bacteria but, so far as the investigations have gone, similar activities for animals.

The discovery that the animal microorganism, *Tetrahymena geleii*, requires pteroylglutamic acid for growth<sup>2</sup> makes it of importance and interest to investigate the activity of the related compounds in the growth of this organism. Because of the fact that the metabolism of *T. geleii* has been shown to be more typically animal-like than that of any other microorganism so far studied,<sup>3</sup> it might be suspected that the two conjugates would prove active. Bacteria<sup>4</sup> differ from typical animals (chick, rat, monkey<sup>5</sup>) in what has been interpreted to be a lack of specific enzymes for degradation of the conjugated compounds. It has been found also that humans suffering from pernicious anemia, unlike normal humans, are unable to utilize the conjugates,<sup>6</sup> and this has also been attributed to a lack of conjugase.

*Material and Methods.*—The organism used in this investigation was the ciliated protozoan, *Tetrahymena geleii* W, grown in pure (bacteria-free) culture.<sup>3</sup> Dose-response tests were carried out as previously described.<sup>3, 7</sup> The base medium has been modified in respect to the amino acid concentrations from that which was formerly used,<sup>3, 7</sup> as our investigations (still



in progress) have shown that better growth can be obtained by such changes. The complete base medium is given in table 1. It is to be noted that both *p*-aminobenzoic acid and inositol have been omitted from this medium, since the organism has been found to be capable of synthesizing these compounds and their inclusion in the medium is without effect.<sup>8</sup> Guanylic acid has been substituted for guanine because of the greater solubility of the former. Brucine cytidylate was used in place of cytidylic acid because our supply of the free acid was exhausted. Comparative tests of these last compounds showed no indications of toxicity of the brucine salt.

TABLE 1

BASE MEDIUM			
	γ/ml.		γ/ml.
<i>l</i> (+) Arginine Monohydrochloride	125	Biotin (free acid).....	0.0005
<i>l</i> (-) Histidine.....	125	Calcium pantothenate.....	0.10
<i>dl</i> -Isoleucine.....	125	Thiamine Hydrochloride.....	1.00
<i>l</i> (-) Leucine.....	250	Nicotinamide.....	0.10
<i>l</i> (+) Lysine.....	250	Pyridoxine Hydrochloride.....	0.10
<i>dl</i> -Methionine.....	500	Riboflavin.....	0.10
<i>dl</i> -Phenylalanine.....	350	Choline chloride.....	1.00
<i>dl</i> -Serine.....	250	Guanylic Acid.....	25.00
<i>dl</i> -Threonine.....	125	Adenylic Acid.....	25.00
<i>l</i> (-) Tryptophane.....	50	Brucine cytidylate.....	50.00
<i>dl</i> -Valine.....	125	Uracil.....	50.00
Dextrose.....	1000	Factor II preparation from liver 1:10	
MgSO <sub>4</sub> ·7H <sub>2</sub> O.....	100	(prepared as previously described <sup>3</sup> )	
K <sub>2</sub> HPO <sub>4</sub> .....	100		
CaCl <sub>2</sub> ·2H <sub>2</sub> O.....	50		
FeCl <sub>3</sub> ·6H <sub>2</sub> O.....	1.25		
MnCl <sub>2</sub> ·4H <sub>2</sub> O.....	0.05		
ZnCl <sub>2</sub> .....	0.05		

Pteroylglutamic acid, pteric acid, pteroyldiglutamyl glutamic acid (fermentation factor) and 2 amino-4 hydroxy-6 methyl pteridine were supplied by Dr. E. L. R. Stokstad of Lederle Research Laboratories. Xanthopterin and *p*-aminobenzoyl-glutamic acid were supplied by Dr. George H. Hitchings of the Wellcome Laboratories. The sample of pteroylhexaglutamylglutamic acid (Vitamin B<sub>6</sub> conjugate) was sent to us by Dr. O. D. Bird of Parke, Davis Co. Research Laboratories. Without the generous coöperation of these investigators this study would not have been possible.

Since the terminology of the group of compounds under discussion is rather cumbersome, a set of abbreviations similar to that used by Lampen and Jones,<sup>9</sup> has been devised. These are as shown in table 2.

TABLE 2

PAB.....	<i>p</i> -Aminobenzoic Acid
PGA.....	Pteroylglutamic Acid
PABG.....	<i>p</i> -Aminobenzoylglutamic Acid
PHGA.....	Pteroylheptaglutamic Acid (this is, in turn, an abbreviation for pteroylhexaglutamylglutamic acid <sup>b</sup> )
PTGA.....	Pteroyltriglutamic Acid (Pteroyldiglutamylglutamic acid <sup>b</sup> )

Results.—When the activity of PGA was tested, using the present base medium, it was found that half-maximum response was obtained with 0.00064 microgram per ml. of medium (Fig. 1). This is in good agreement with our earlier findings<sup>7</sup> of 0.00065 microgram per ml. of medium for half-maximum activity.

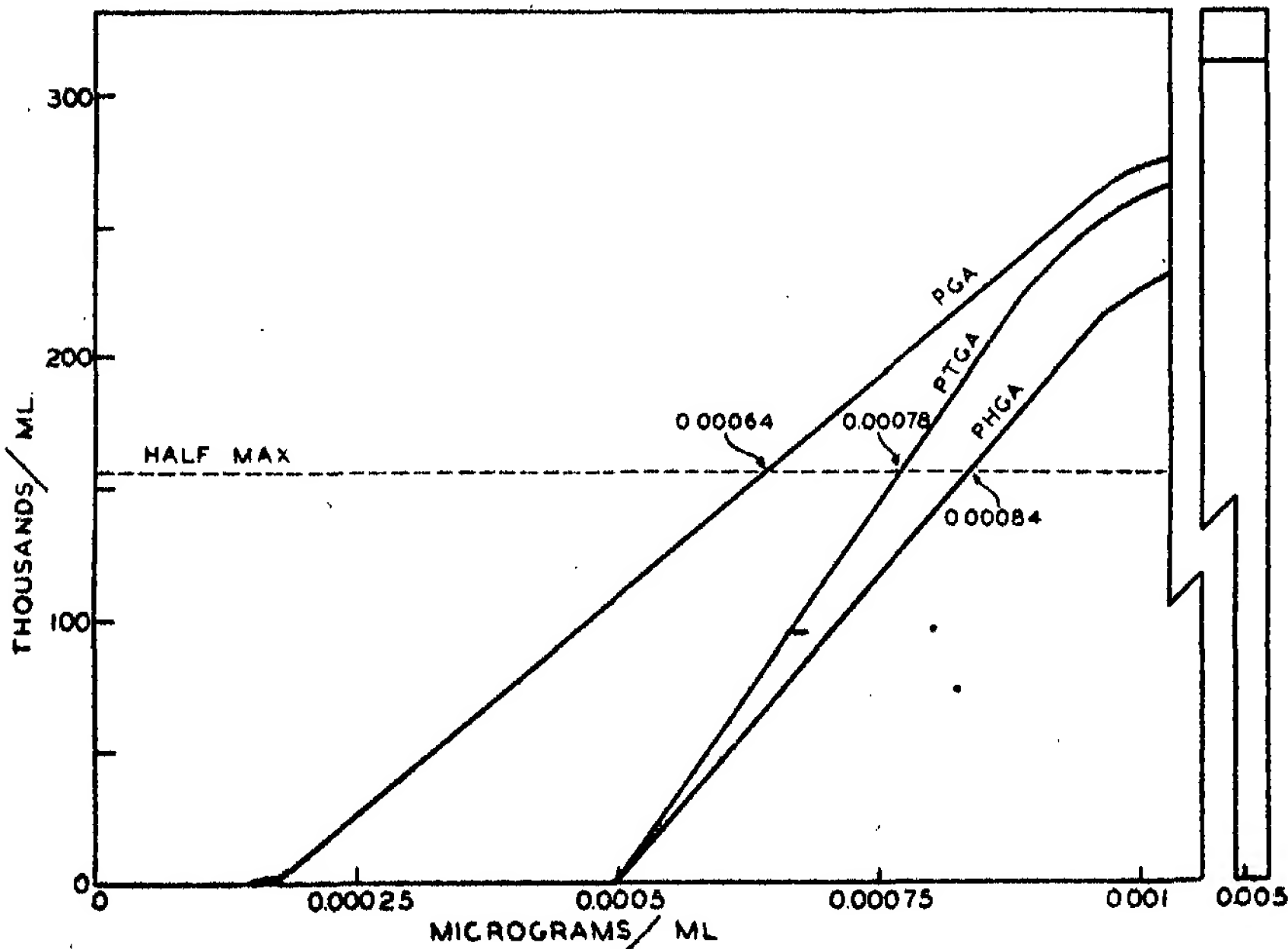


FIGURE 1.

Dose response curves of PGA, PTGA and PHGA. Dosage is on a total weight of compound basis. The curves were constructed from results obtained after third serial transplant.

Figure 1 also represents the growth response of *T. geleii* to varying concentrations of PTGA and PHGA. The half-maximum potency of PTGA is 0.00078 microgram per ml. and that of PHGA is 0.00084 microgram per ml. These responses are on the basis of the total weight of compound used. When these values are recalculated for the free PGA content, very

different curves are obtained (Fig. 2). It is immediately apparent that PHGA is more active than PTGA, which, in turn, is more active than the free acid. On the basis of PGA content, PHGA has a half-maximum potency of 0.00032 microgram per ml. This figure is just half of that which is obtained for free PGA, indicating that in the conjugated form PGA is twice as active. The half maximum activity of PTGA may be expressed as 0.0005 microgram of free PGA per ml., which is intermediate between the two.

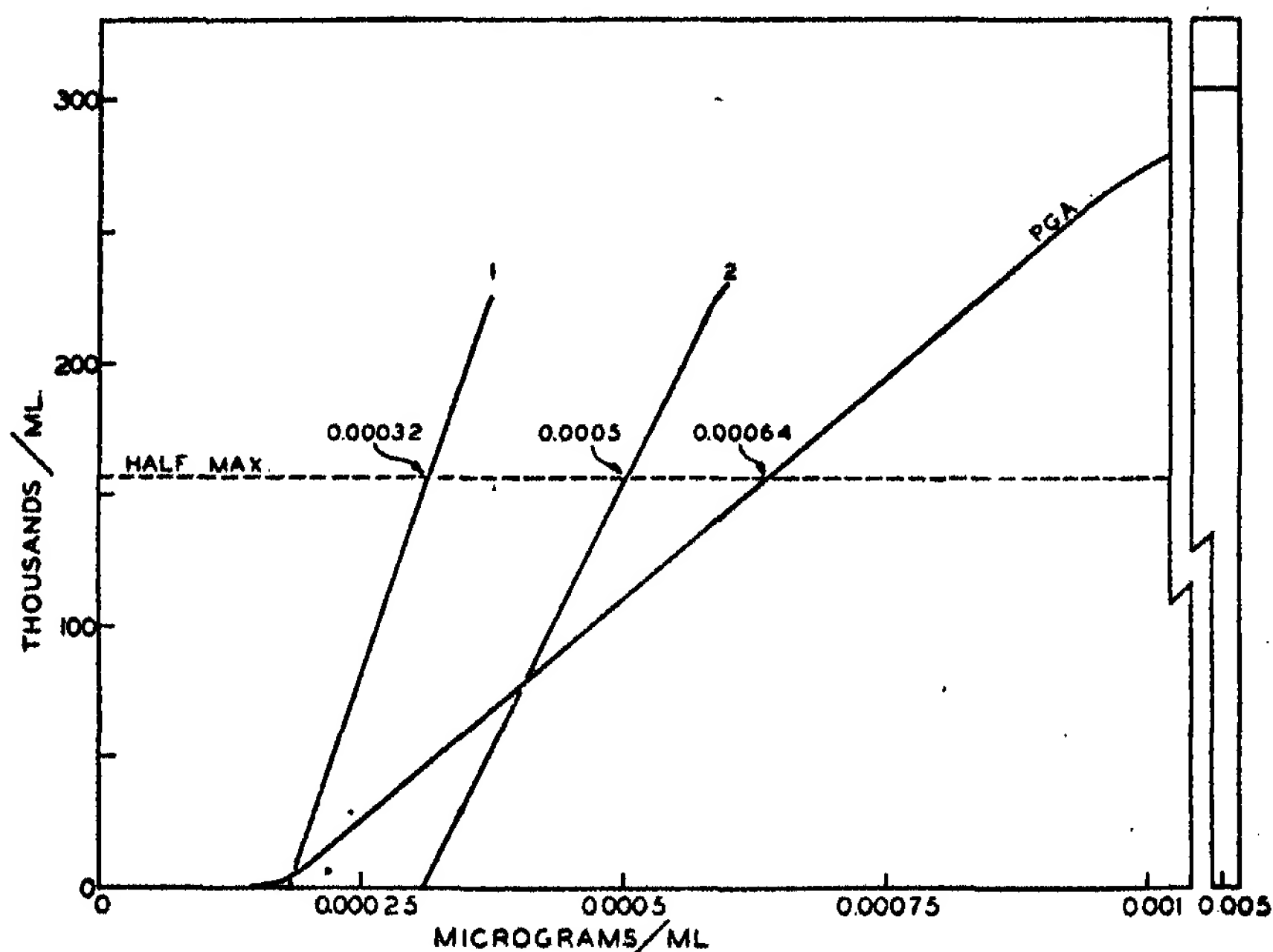


FIGURE 2.

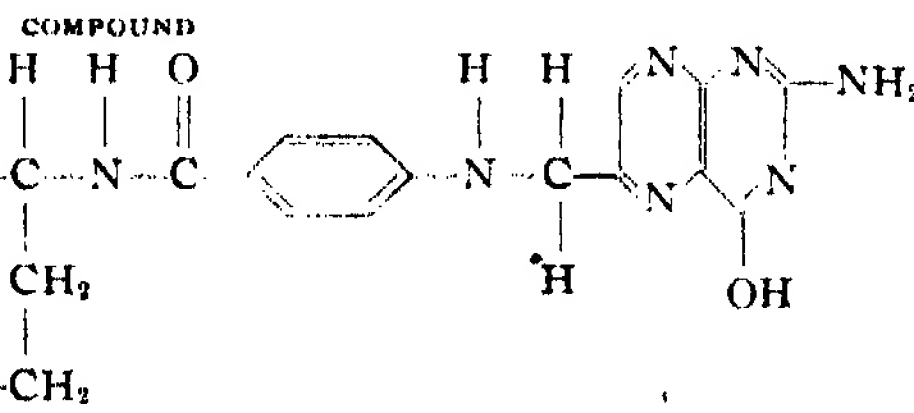
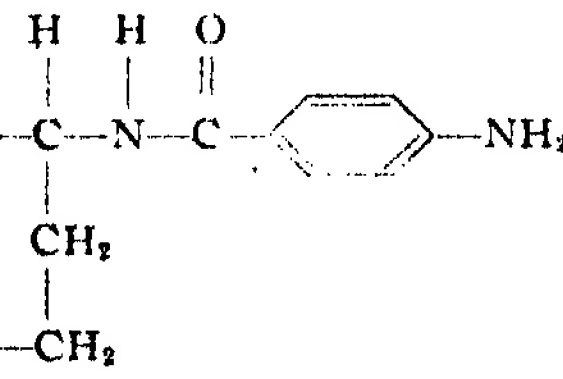
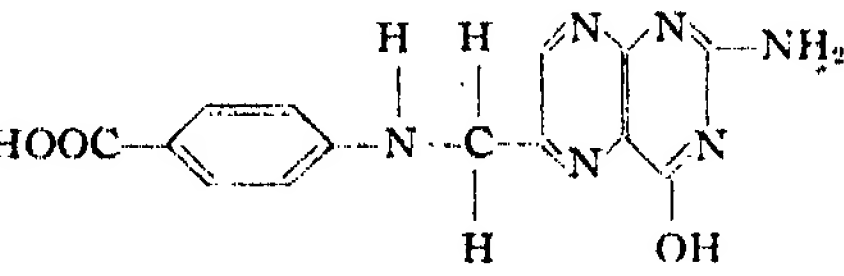
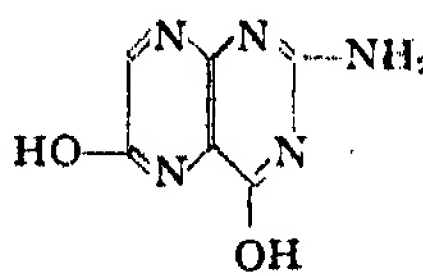
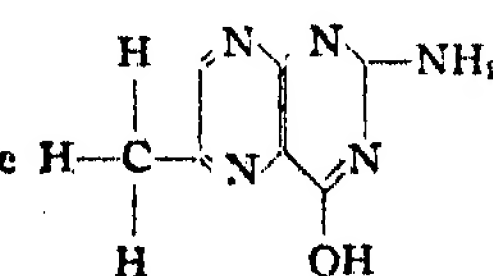
Dose response curves calculated from same data as that used in Figure 1. In this series of curves all values represent the amount of pteroylglutamic acid present. Curve 1 is for PHGA. Curve 2 is for PTGA.

Inasmuch as our previous findings<sup>7</sup> had shown that the SLR factor, which is fully active for *Streptococcus fecalis* and inactive for *Lactobacillus casei*, had low but definite activity for *T. gelei*, it was of importance to test the activity of synthetic pteric acid, which is fully active for *S. fecalis* and inactive for *L. casei*. Pteric acid proved to be entirely without activity in concentrations as high as 0.5 microgram per ml., either with or without added glutamic acid. By comparison with the SLR factor,<sup>7</sup> this amount should have been adequate for half-maximum growth of *T.*

*geleii* if the two are identical. No toxicity of the pterioic acid was noted at this level.

Xanthopterine and 2 amino-4 hydroxy-6 methyl-pteridine were without activity, either alone or in combination with PABG. These results are summarized in table 3.

TABLE 3  
GROWTH RESPONSE OF *Tetrahymena geleii* W TO VARIOUS COMPOUNDS OF THE "FOLIC ACID" GROUP

COMPOUND		1/2 MAX. ACTIVITY 7 ML.
PGA		0.00064
PTGA (expressed as PGA content)		0.0005
PHGA (expressed as PGA content)		0.00032
PABG		Not active
Pterioic Acid		Not active
Xanthopterine		Not active
Methyl Pteridine		Not active
Pterioic Acid + Glutamic Acid		Not active
Xanthopterine + PABG		Not active
Methyl Pteridine + PABG		Not active

*Discussion.*—The fact that synthetic pteric acid fails to give growth at levels at which the SLR factor does, would at first thought appear to indicate their non-identity. An earlier report to this effect has been published.<sup>8</sup> However, when it is recalled that the sample of SLR used is a concentrate from natural sources, rather than a synthetic product, it would appear likely that a slight contamination with PGA was present. This would readily account for the results, since the amount of SLR factor required for half-maximum growth was about 500 times as great as the amount of PGA required. Thus, the presence of 0.02 per cent PGA in the sample would be sufficient to give the results obtained.<sup>7</sup> It may be, therefore, that the SLR factor and pteric acid are identical.

The results with pteric acid also indicate that *T. geleii* is unable to form the specific peptide bond which links glutamic acid to the PAB portion of the pteric acid molecule in the formation of PGA.

Similarly it appears that this ciliate cannot join a pteridine ring to the amino group of either free PAB or of PABG. When xanthopterin was found to be inactive, the possibility still remained that a pteridine with a methyl or hydroxymethyl group in position 6 would exhibit activity. The failure to synthesize PGA from PABG and xanthopterin might have been due to an inability to transmethyrate either the PABG or the xanthopterin. Whether or not this transmethylation is possible cannot be decided, since the organism also failed to synthesize PGA from PABG and the methyl-pteridine. It would be of interest to test its ability to utilize *p*-methylinino-benzoyl glutamic acid and xanthopterin, but the former compound was not available.

*Tetrahymena geleii* is the only microorganism which has so far proved capable of utilizing PHGA. The failure of other microorganisms to utilize PHGA has been attributed to a lack of carboxy-peptidase.<sup>6</sup> Since by far the larger part of the naturally occurring vitamin is in the conjugated form, *T. geleii* should be valuable as an assay organism. Dr. O. D. Bird is at present investigating the formulation of such an assay procedure. The occurrence of PGA largely in the conjugated form in natural foodstuffs is of great theoretical as well as practical importance. It is this fact which explains the failure of patients with pernicious anemia to recover on a natural diet. Free PGA causes an immediate remission of the symptoms, while PHGA is without effect.<sup>6</sup> This has been attributed to the fact that in such patients the enzyme (carboxy-peptidase) responsible for splitting the conjugated form to free PGA is lacking.<sup>10</sup>

In macrocytic anemias due to nutritional deficiencies the situation is different, since any one of the three compounds, PGA, PTGA, or PHGA, is effective.<sup>11</sup> Whether or not activity increases with the addition of more and more glutamic acid residues as it does in the case of *T. geleii*, remains to be seen, although there seems to be some indication that this may be

so. Totter<sup>11</sup> reports that PTGA gives a better and more lasting response than equal amounts of PGA. On the other hand, Hutchings, *et al.*,<sup>12</sup> report equal activity for PTGA and PGA in producing growth and stimulating hematopoiesis in the chick, but they concluded that biological variability in these experiments makes it difficult to assess the comparative activity.

It is likewise difficult to find an explanation for the greater activity of PHGA. In other microorganisms (e.g., *L. casei*) this compound is practically devoid of activity, indicating a lack of conjugase. It seems evident that other organisms also require at least a part of the vitamin in the free form. In addition it is apparent that at least part of the PGA is stored in the conjugated form. It is known that *T. geleii* stores relatively large amounts of the PGA with which it is supplied.<sup>2</sup> It is therefore possible that stores of PGA as PHGA must first be replenished, while free PGA is utilized in metabolism. Piffner<sup>5</sup> makes the statement that PHGA is utilized directly in metabolism, but does not offer experimental support for this statement. That free PGA must be required at least in part is evidenced by the results on pernicious anemia patients. It is also possible that free PGA is required for hematopoiesis and not for other metabolic functions. If it is true that storage of PHGA is compulsory, then, when only free PGA is supplied, storage would be less efficient because synthesis of both glutamic acid and of PHGA are involved. When PHGA is supplied storage proceeds immediately and only a small portion need be hydrolyzed for metabolic needs. This would also explain the intermediate position of PTGA in the scale of potency. Effectiveness of the various compounds appears to increase directly with the number of glutamic acid residues present.

*Summary.*—Vitamin B<sub>6</sub> conjugate (pteroylhexaglutamylglutamic acid) is utilized by the ciliate *Tetrahymena geleii* W. On the basis of its pteroylglutamic acid content it is twice as active as the free acid. Fermentation factor (pteroyldiglutamylglutamic acid) is utilized and its activity is intermediate between the other two compounds, on the basis of its pteroylglutamic acid content. Xanthopterin, *p*-aminobenzoyl glutamic acid, pteric acid and a methyl pteridine proved to be inactive, either alone or in combination.

\* Aided by a grant from the U. S. Public Health Service.

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<sup>7</sup> Kidder, G. W., and Fuller, R. C., *Science*, **104**, 160 (1946).

<sup>8</sup> Kidder, G. W., *Ann. N. Y. Acad. Sci.* (in press) (1947).

<sup>9</sup> Lampen, J. O., and Jones, M. J., *Jour. Biol. Chem.*, **166**, 435 (1946).

<sup>10</sup> Welch, A. D., *et al.*, *Ann. N. Y. Acad. Sci.*, **48**, 347 (1946).

<sup>11</sup> Totter, J. R., *Ibid.*, **48**, 309 (1946).

<sup>12</sup> Hutchings, B. L., Oelson, J. J., and Stokstad, E. L. R., *Jour. Biol. Chem.*, **163**, 447 (1946).

## BLOOD GROUPS OF THE RAT (*RATTUS NORVEGICUS*) AND THEIR INHERITANCE

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Earlier studies of rat blood were largely exploratory. Rohdenberg (1920) was unable to detect agglutination in mixtures of blood from 50 rats. Lambert (1927) failed to find agglutination in blood mixtures from 46 rats of five different strains. Dr. Hibino, however, working with Dr. Furuhashi, an authority on the blood groups of the Japanese, demonstrated isohaemagglutination in the rat, but did not continue the work (personal communication in 1934). Friedberger and Taslokawa (1928) found numerous cases of agglutination between the blood of wild and tame rats in Berlin, and postulated four haemagglutinins with corresponding agglutinogens.

My own studies were begun at the Bussey Institution of Harvard University in 1932, at the suggestion of Dr. W. E. Castle, and were continued subsequently at the University of Maryland.

Blood group differences in animals depend upon the existence of two complementary agencies in blood, an agglutinogen carried in blood cells, and an agglutinin carried in blood plasma. Clumping of the blood cells occurs when the two agencies are brought together. Blood which contains a particular agglutinogen regularly lacks the corresponding agglutinin; otherwise it would clump spontaneously.

Consequently agglutination occurs only when blood from different individuals is mixed, one supplying the agglutinogen, the other the agglutinin.

Animals of a species may be classified in blood groups on the basis of their possession or lack of particular agglutinogens.

In a search for blood groups in a species in which their existence is un-

certain, it is desirable to make combinations of blood from as many unrelated stocks as possible. In the present investigation rats were obtained for study from 15 different laboratory stocks, in addition to wild rats caught at Forest Hills, Mass.

In the course of the investigation, two agglutinogens have been found, one of which resembles the A and B agglutinogens of human blood, the other resembling the M and N agglutinogens of human blood. Because of these resemblances it seems appropriate to designate the newly discovered agglutinogens of the rat A and M respectively.

The agglutinin which acts in conjunction with agglutinin A to effect blood clumping is found as a natural ingredient of the blood serum of *all rats which do not possess the A agglutinin*. It may be called agglutinin *a*.

An agglutinin, which will act in conjunction with agglutinin M to induce blood clumping, does not exist as a natural ingredient of rat blood, at least not in detectable amounts. But it can be artificially produced by injections of blood containing agglutinin M into animals which lack M. The agglutinin, which may be called *m*, is produced as an antibody to the foreign substance, M. Blood serum containing such an antibody is called an immune serum.

To secure blood for injection or for agglutination tests, the end of the tail may be snipped off, the animal being first etherized. But the yield by this method is small, usually 2 cc. or less, and the product frequently contaminated.

A better method is to bleed from the heart, as described by Burhoe, 1940. The yield is larger and more likely to be sterile.

To obtain serum, the blood is collected in 6 cc. agglutination tubes and allowed to clot. The clot is broken up with a clean probe or small twisted wire, and the material centrifuged.

In preparation for testing the agglutinating properties of a rat's blood corpuscles, a few drops of freshly drawn blood are put in a mixture of 1% sodium citrate in physiological salt solution.

Injections for the production of an immune serum may be made either subcutaneously or into the body cavity. An injection of from 1 cc. to 5 cc. of blood should result in producing immune agglutinin in about five days. The immunity, however, gradually disappears thereafter and is entirely gone within two months, unless the injection is repeated.

The presence of agglutinin *a* in a rat does not preclude the development of agglutinin *m* along with it. Thus a rat possessing *a* may be made to develop *m* also, if agglutinin M is injected into it, resulting in bivalent serum, *a + m*.

In the initial experiments mixing of blood from different laboratory stocks gave negative results (no clumping) as a like procedure had in the case of many earlier investigators, but finally a stock of red-eyed yellow

rats supplied by Dr. H. W. Feldman of the University of Michigan, showed clumping of blood cells introduced into its serum from all other races tested. This result indicated that the yellow race contained in its serum an agglutinin which was effective in the clumping, but was an exclusive possession of that particular race.

This race was the original source of agglutinin *a*. Its serum was used in diagnosing the blood constitution (presence or absence of agglutininogen A) of other laboratory stocks and of captured wild rats. In fact all animals so tested in the initial experiments were found to have A. But when crosses were made between the peculiar red-eyed yellow race and other stocks, there appeared in the  $F_2$  generation an abundance of animals (recessives) which lacked the A agglutininogen and so naturally possessed the *a* agglutinin.

The existence of a second agglutininogen was demonstrated by the method, originally devised by Landsteiner, of producing immune sera by reciprocal or multiple exchanges of blood between individuals. In exploratory studies of the rat being undertaken in this case, the method is particularly effective when races differing as widely as possible are used. The immune serum *m* was produced thus. Blood was taken from a selected individual of each of eleven different laboratory stocks. Two cc. of blood were drawn from each donor, centrifuged to separate the blood cells, which were then washed in saline, pooled and injected intraperitoneally at semi-weekly intervals into an individual of each of the eleven races which had furnished the blood cells. Tests for the presence of an agglutinin were thereafter made every two weeks, using serum of each injected animal, into which pooled blood cells of the donors were introduced.

After six weeks of injections the immune agglutinin was detected in the sera of certain of the injected animals, its presence being revealed by clumping of the introduced blood cells. For example, serum of the injected individual of family D was found to clump cells of families E, H, and J. Further tests made with the newly produced serum, showed that of the 16 families included in the study, 9 laboratory stocks consisted wholly or in part of individuals carrying the M agglutininogen, while the wild rats tested all were carriers of it. Four different albino stocks, including two Wistar albino strains, and a black strain supplied by Dr. Feldman were found to lack the M agglutininogen but to carry A. Only one family, the red-eyed yellow family supplied by Feldman, carried neither agglutininogen.

Demonstration of the existence of two different agglutinogens in the rat make it possible to classify individuals in four blood groups, viz., (1) those which carry both A and M (group AM), (2) those which carry A but not M (group A), (3) those which carry M but not A (group M) and (4) those which carry neither A nor M (group O).

The results of crosses made between individuals of the four blood groups

are summarized in table 1. They show the character of each individual tested with diagnostic sera in 508 litters of rats aggregating 3203 individuals. The crosses show consistently that the agglutinogens are inherited as dominant characters and assort independently, which means that their genes are carried in different chromosome pairs.

TABLE 1  
DATA ON THE INHERITANCE OF THE TWO BLOOD GROUP GENES, A AND M

CROSS NO.	GENOTYPE OF PARENTS	NO. OF LITTERS	BLOOD GROUPS OF YOUNG				TOTAL YOUNG
			AM	A	M	O	
1	O × O	9	...	...	...	53	53
2	AAMM × AAMM	10	61	...	...	...	61
3	AA × AA	19	...	93	...	...	93
4	AAMM × O	16	92	...	...	...	92
5	AM × AM	23	88	32	33	10	163
6	AM × O	50	102	85	74	89	350
7	AMM × O	4	15	...	13	...	28
8	A × M	14	29	21	16	21	87
9	AAM × O	6	28	25	...	...	53
10	M × M	16	...	...	78	32	110
11 <sup>a</sup>	AA × O	8	...	50	...	...	50
11 <sup>b</sup>	F <sub>2</sub> , A × A	24	...	100	...	42	142
11 <sup>c</sup>	BC, A × O	90	...	283	...	262	545
12 <sup>a</sup>	MM × O	11	...	...	61	...	61
12 <sup>b</sup>	F <sub>2</sub> , M × M	43	...	...	177	71	248
12 <sup>c</sup>	BC, M × O	89	...	...	282	293	575
13 <sup>a</sup>	AA × MM	2	7	...	...	...	7
13 <sup>b</sup>	F <sub>2</sub> , AM × AM	19	65	24	23	7	119
14 <sup>a</sup>	AAMM × O	14	80	...	...	...	80
14 <sup>b</sup>	BC, AM (F <sub>1</sub> ) × O	41	77	70	64	75	286
	Totals	508	...	...	...	...	3203

From an examination of table 1 the following conclusions may be drawn:

1. Group O individuals are double recessives, carry neither dominant gene, and breed true (cross 1).

2. Group A individuals may be either homozygous (AA) or heterozygous (A). When homozygous they produce only group A progeny in matings with group O individuals (cross 11<sup>a</sup>). When heterozygous they produce both A and O individuals in a 1:1 ratio (cross 11<sup>c</sup>).

3. Group M individuals also may be either homozygous (MM) or heterozygous (M). When homozygous they produce only group M progeny in matings with group O individuals (cross 12<sup>a</sup>). When heterozygous they produce both M and O progeny in a 1:1 ratio (cross 12<sup>c</sup>).

4. Group AM individuals carry both dominant genes (A and M) but may be either homozygous for both, heterozygous for one only or heterozygous for both. The four possible varieties of genetic constitution of group AM individuals are shown in the following crosses: AAMM in

crosses 4 and 14<sup>a</sup>; AAM in cross 9; AMM in cross 7; AM in crosses 6 and 14<sup>b</sup> (in which a 1:1:1:1 ratio is approximated).

5. When double heterozygotes (AM) are mated together, a 9:3:3:1 ratio is obtained (crosses 5 and 13<sup>b</sup>).

We may conclude that the inheritance of the two agglutinogens, A and M, is in every respect typically mendelian, that the two dominants assort independently and so are undoubtedly carried in different chromosome pairs.

*Linkage Studies*—In order to identify, if possible, the chromosome pairs in which the genes for the agglutinogens A and M are located, tests have been made for linkage with eight different mutant genes of the rat, each of which is thought to be located in a different chromosome pair. The results of these tests are summarized in tables 2 and 3.

TABLE 2

DATA FROM TESTS FOR LINKAGE BETWEEN THE GENE FOR AGGLUTINOGEN A AND EIGHT OTHER MUTANT GENES OF THE RAT. D MEANS THE DOMINANT ALLELE OF THE CHARACTER UNDER INVESTIGATION, O MEANS DOUBLE RECESSIVE

MUTANT GENE AND NATURE OF CROSS	CLASSES OF YOUNG				TOTAL YOUNG	CROSS- OVERS	NON-CROSS- OVERS	DEV/PM
	DA	D	A	O				
Agouti, R	23	21	25	22	91	45	46	0.1
Kinky, R	25	27	21	22	95	47	48	1.5
Red-eye, R	14	11	16	17	58	31	27	0.8
Curly, C	14	14	15	16	59	29	30	0.1
Curly <sub>2</sub> , C	22	20	20	22	84	40	44	0.6
Blue, C	19	20	15	21	75	35	40	0.9
Hairless, C	39	30	39	38	146	69	77	0.9
Hooded, F <sub>2</sub> , R	97	30	30	9	166	60 (expected 62)		0.5

Tests for linkage of A are shown in table 2. Crosses were first made to produce individuals doubly heterozygous for gene A and one of the mutant genes under investigation. Then the double heterozygote was crossed to the appropriate double recessive, if such was available. In table 2, the first seven crosses listed were of this nature, a double heterozygote being mated to a double recessive. If the original cross was *repulsional*, this is indicated by R in the table, first three and last entry. If the cross was coupling in character, this is indicated by C, four entries. The classes of young which are *crossover* (recombinations) are italicized.

In the case of the hooded gene, no double recessive was available for crossing with the double heterozygote. Consequently an F<sub>2</sub> population was produced. In this case the two middle classes of young, numbering 30 each, are recombination classes which would involve, in the production of every individual, either one or two crossover gametes. If there is no linkage (repulsion) between A and the gene for hooded, we should expect the middle classes to contain ten-sixteenths of the population of 166 individuals,



i.e., 62. Actually they contain 60, a deviation without statistical significance.

If there were linkage, we should expect a significant diminution in these two classes from the calculated total, 62. Since it does not occur, it is fair to conclude that existence of linkage is highly improbable in this case.

The seven other linkage tests recorded in table 2 were made by the preferable method of crossing an  $F_1$  individual to double recessive mates. In each instance an equality of crossover and non-crossover individuals is expected, and from this expectation no significant deviation is observed, as shown in the last column of the table.

TABLE 3

DATA FROM TESTS FOR LINKAGE BETWEEN THE GENE FOR AGGLUTINOGEN M AND EIGHT OTHER MUTANT GENES OF THE RAT. D MEANS THE DOMINANT ALLELE OF THE CHARACTER UNDER INVESTIGATION, O MEANS DOUBLE RECESSIVE

MUTANT ORIGIN AND NATURE OF CROSS	CLASSES OF YOUNG				TOTAL YOUNG	CROSS- OVERS	NON-CROSS- OVERS	DEV/PE
	DM	D	M	O				
Agouti, C	34	35	42	31	142	77	65	1.5
Curly, C	14	14	14	17	59	28	31	0.6
Curly <sub>2</sub> , C	18	26	20	22	86	46	40	0.9
Hairless, R	19	17	11	12	59	31	28	0.4
Hooded, R	34	27	35	28	124	62	62	0
Blue, $F_2$ , C	50	17	19	5	91	5 (expected 5.7)		0.4
Kinky, $F_2$ , R	81	33	15	6	135	48 (expected 50.6)		0.7
Red-eye, $F_2$ , R	67	23	18	8	116	41 (expected 43.5)		0.7

In table 3 tests for linkage of the same eight mutant genes with M are recorded. Here are shown the results of three original coupling crosses subsequently back-crossed to the double recessive (first three entries). There follow two repulsion crosses similarly back-crossed to the double recessive. Finally listed are three  $F_2$  populations from crosses, one of which involved the coupling relationship, and the last two the repulsion relationship. No indication of linkage is found in the five back-cross experiments.

The  $F_2$  tests for kinky and red-eye (last two entries) are similar in character to the hooded test in table 2 already discussed and have a similar outcome. No significant deviation is shown from the numbers expected in the two middle (exclusively crossover) classes, if no linkage exists.

The  $F_2$  test for blue was based on a coupling cross. Here the double recessive class (4th column) could arise only from recombination (crossover) gametes. Its frequency is 5, where the maximum expectancy, if no linkage exists, is 5.7, a non-significant difference.

We may conclude that the experiments summarized in tables 2 and 3 give no indication of linkage between the genes for agglutinogens A and M and genes serving as genetic markers of eight pairs of autosomes of the rat.



Since we must conclude that the genes for A and M do not lie in any of the eight chromosome pairs tagged by the mutant genes listed in tables 2 and 3, it follows that they will constitute marker genes, respectively, for a 9th and 10th autosomal pair. Dr. Castle informs me that in recent years the number of known mutant genes has been increased to 22. It is quite possible that some of the newly discovered mutant genes, not listed in tables 2 and 3, may actually lie in chromosomes for which agglutinogens A and M now serve as markers. To ascertain this, further linkage studies are needed.

*Summary.*—1. In an attempt to discover blood groups in the rat, 15 different laboratory stocks and a collection of wild rats have been studied.

2. The wild rats and ten of the laboratory stocks were found to be carriers of two different agglutinogens, A and M. Four of the laboratory stocks carried A only, and one stock carried neither A nor M.

3. Rats which lack A, either by original mutation or by genetic recombination following a cross with a race lacking A, have as a natural ingredient of their serum an agglutinin which will cause clumping of the blood corpuscles of a rat having agglutinogen A. This natural agglutinin may be called agglutinin *a*.

4. Agglutinogen M is capable of demonstration only by immune serum created by injection of blood containing M into rats which lack it. Such an artificially induced agglutinin may be called *m*. It causes agglutination of blood cells containing M, when they are introduced into it.

5. On the basis of the presence in or absence from individual rats of the agglutinogens A and M, rats may be classified in four blood groups, AM, A, M and O. Wild rats (so far as studied) and many laboratory stocks (Long-Evans, at least in part) are AM. Most stocks of albino rats (including Wistar stock albinos) are A, as are also some colored stocks. One stock only has been found to be O. An M group has been obtained as an F<sub>2</sub> recombination class, following a cross between AM and O individuals.

6. Presence of Agglutinogen A or agglutinin *a* in a rat does not interfere with the development also in it of agglutinin *m*, upon injection of M cells into said rat. A bivalent test serum results, *a* and *m*.

7. Agglutinogens A and M are inherited as simple dominant characters, and may occur either as homozygotes or as heterozygotes, together or apart. They segregate and recombine independently, and behave in every respect as typical autosomal characters.

8. No indications were found of linkage of either A or M with eight mutant genes believed to be borne in as many different chromosome pairs.

\* Based in part on a thesis for the degree of Ph.D. presented to the Biological Faculty of Harvard University in 1937.

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## THE DOMESTICATION OF THE RAT

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The Norway rat (*Rattus norvegicus*) is a comparatively recent immigrant to Europe and America and at the present time is reproducing in enormous numbers both in a wild state and under domestication. A comparative study of its behavior in the two contrasted states is thus made easy and should throw light on what takes place in a species of mammal when it is brought into captivity and its breeding is controlled by man.

The Norway rat entered western Europe by way of the Norwegian peninsula in the first half of the Eighteenth Century and bears a specific name indicating the route by which it arrived. Its ecological predecessor was the black rat (*Rattus rattus*) the rat which spread plague in London in 1664-1665. This species was soon afterward replaced in Western Europe by its mortal enemy the newly introduced Norway rat, which promptly made its way on ships to the New World, where the black rat had preceded it but was, as in Europe, promptly supplanted by the Norway rat except in out-lying districts such as northern New Hampshire where Dr. C. C. Little secured for me live examples of *Rattus rattus* about 1910, and on which Dr. H. W. Feldman made genetic studies. One interesting result of these studies was the demonstration that crosses between the two species, *R. rattus* and *R. norvegicus*, are very difficult to obtain: embryos never coming to term alive. So it is easy to see why hybrids do not occur in nature.

At sometime after the introduction of the Norway rat into Western Europe, it is probable that albino mutants made their appearance in the

wild population, as they do in the case of most wild mammals. Albino individuals were probably captured and tamed because of their attractive and distinctive appearance and thus the tame white rat became the earliest domesticated type of rat. Similarly a black (non-agouti) mutation made its appearance, and also a piebald (hooded) mutant appeared, perhaps originally gray-and-white in color, but if mated to black individuals resulting in the production of the double recessive black hooded type. Then if the black hooded type was crossed with albinos, triple recessives would result, albinos homozygous for black and piebald ( $cc\ aa\ hh$ ). Keeler has shown that black individuals are in temperament gentler than grays of like ancestry and it seems probable that this made easier the process of taming the mutant strains, since the gentler individuals would be more amenable to handling and confinement and would thus come to predominate in the domesticated race.

At any rate we know that when the first recorded breeding experiments with white rats were undertaken by Crampe about 1880, an albino female which he mated to a wild gray male transmitted as recessives to her gray  $F_1$  offspring the three mutant genes,  $c$  (albino),  $a$  (non-agouti), and  $h$  (hooded). Although Crampe's experiments were made in the pre-Mendelian period, his records as analyzed by Doncaster (1906) fully support this interpretation.

It is clear also that when white rats of European origin were brought to America (by Dr. H. H. Donaldson and others) and were made the foundation of the Wistar Institute race of albinos, these were homozygous for the same three mutant genes ( $cc\ aa\ hh$ ) as were Crampe's albino. Albino rats which Dr. Donaldson kindly supplied to me about 1903 were of this genetic constitution.

Dr. Donaldson in 1906 transferred his colony of albino rats from the University of Chicago to the Wistar Institute. Here Dr. Helen Dean King in 1908 became associated with him in a comparative study of the albino rat and its wild gray ancestor. She had the happy thought that it would be interesting to re-enact the domestication of the rat under controlled conditions and thus to observe just what occurs in the process. In this she had the coöperation and support of Dr. Donaldson, with consequences of the highest importance to the science of genetics. In the spring of 1919 Dr. King began rearing in captivity the progeny of wild rats captured in the vicinity of Philadelphia. In 1929 she reported on the life processes observed in the first 10 generations of captive gray rats, Dr. Donaldson at the same time reporting on size of body and organs of the rats. Ten years later, after Dr. Donaldson's death, Dr. King made a further report on life processes as observed in 26 generations of captive rats.

Donaldson in 1929 had summarized as follows the initial differences

between wild gray rats and albinos. "The wild Norway are more excitable and much more savage. They gnaw their cages. The body weight is less for a given body length, hence it is a slighter animal. The skeleton is relatively heavier, also the suprarenals (both sexes) and the testes and ovaries. The thyroid is of like weight, but the hypophysis distinctly lighter in both sexes. On the other hand, the brain and the spinal cord are both heavier than in the Albino."

After ten generations in captivity Donaldson finds that in captive grays there has been an increase in body weight in relation to body length, i.e., the body has become less slender, more like the albino in conformation. The hypophysis has increased slightly in weight. No change has occurred in the weight of the gonads. Decrease in weight is shown by brain, thyroid and suprarenals. But brain, suprarenals, gonads and bones are still heavier than in the Albino race. "Ten generations of captivity have, by no means, he says, served to give the captive Grays the organ constitution of the Albino." It would seem, accordingly, that a changed and controlled environment had effected little racial change in the course of ten generations.

As regards the changes observed in life processes during 25 generations in captivity Dr. King (1939) notes a gradual increase in the "rate and extent of body growth," i.e., in general body size. "At the last generation growth acceleration (more rapid growth during the adolescent period) was nearly equal to that found in stock albino rats that have been under domestication for a long period of time. At the twenty-fifth generation adult rats of both sexes were, on the average, about 20 per cent heavier than individuals of the first generation."

"Rats attaining an adult weight much above the average for all individuals of like sex in the same generation group appeared in increasing numbers as the generations advanced." The weight increase is ascribed tentatively to genetic mutation rather than to a direct effect of a changed environment.

At the twenty-fifth generation the average length of the reproductive period was nearly 8 months longer than for the first generation. This extension resulted from the earlier breeding of the rats and the persistence of reproduction to a more advanced age.

Fertility of the rats, as measured by litter production increased steadily reaching its maximum at the nineteenth generation where females produced an average of 10.18 litters each, as contrasted to an average of 3.5 litters each for generation one. No significant change in the size of individual litters was observed. Litter size continued at an average of 6.1 throughout generations 2-26. Variability in body size decreased, i.e., the race became more uniform in body size.

On the whole it would seem that in the experiments of Dr. King genetic

differences present in the foundation animals or mutational changes occurring in their descendants will account adequately for the changes observed.

Those changes are (1) accelerated growth rate resulting in increased body size; (2) decreased "nervous tension" resulting in tamableness when the animals were handled frequently in early life; (3) mutations in color or structure of the hair.

In the course of Dr. King's experiments with captive wild gray rats, she observed the new occurrence among them of four mutations previously known, *c*, *a*, *h* and *c<sup>d</sup>* of table 1. Of these *h* was already present as a re-

TABLE 1  
MUTATIONS OF THE RAT

DESIGNATION	GENETIC SYMBOL AND LINKAGE GROUP	NATURE	TIME AND PLACE OF ORIGIN	RECORDED BY	NATURE OF POPULATION IN WHICH IT APPEARED
1. Albino	<i>c</i> I	Absence of pigment from coat and eyes	17th or 18th Centuries in Western Europe	H. Crampe, 1885	Wild
2. Non-agouti	<i>a</i>	Absence of wild coat pattern, uniform black	17th or 18th Centuries in Western Europe	H. Crampe, 1885	Wild
3. Hooded	<i>h</i> I	White except head and back stripe	17th or 18th Centuries in Western Europe	H. Crampe, 1885	Wild
4. Pink-eyed yellow	<i>p</i> I	Coat yellow, eyes pink	1907, England	Castle	Wild
5. Red-eyed yellow	<i>r</i> I	Coat yellow, eyes red	1907, England	Castle	Wild
6. Curly	<i>Cu</i> II	Hairs of coat and vibrissae curved	1920-1930 Wistar Institute	Helen Dean King	Captive wild
7. Brown	<i>b</i> II	Black pigment of coat and eyes replaced by brown	1920-1930 Wistar Institute	Helen Dean King	Captive wild
8. Stub	<i>s</i> IV	Short stubby tail	1939 Wistar Institute	Helen Dean King	Captive wild
9. Ruby-eyed dilute	<i>c<sup>d</sup></i> I	Allele of albino gene, <i>c</i> , pigmentation diminished	1918 in wild rats in Phila.; later in captive grays, 1920-1930	Whiting and King 1918, King 1939	Captive wild

10.	Curly <sub>2</sub>	<i>Cu<sub>2</sub></i>	Coat hairs and vibrissae strongly curved	Davis, Calif., 1935	Blunn and Gregory	Long-Evans captive gray stock
11.	Kinky	<i>k</i> IV	Coat hairs and vibrissae strongly curved	Ann Arbor, Michigan, 1935	H. W. Feldman	Domesticated strain
12.	Lethal	<i>l</i> I	Skeleton imperfect	England 1939	H. Grüneberg	Domesticated strain
13.	Blue	<i>d</i>	Black pigment diluted (clumped) to yield a blue	University of Illinois, 1929	E. Roberts	Domesticated strain
14.	Hairless	<i>hr</i> III	Hair lost at about 4 weeks of age	University of Illinois, 1940	E. Roberts	Domesticated strain
15.	Wobbly	<i>wo</i> III	Ataxic locomotion	Univ. of Iowa, 1941	Castle, King, and Daniels	Domesticated strain
16.	Waltzing	<i>w</i> I	Runs in circles	Wistar Institute, 1936	Helen Dean King	Domesticated strain
17.	Incisorless	<i>in</i> II	Incisors lacking	Squibb Labs., New Brunswick, N. J., 1941	R. O. Greep	Domesticated strain
18.	Anemia	<i>an</i> II	Young anemic at birth, lack of red blood cells	Cornell Univ., 1939	Smith and Bogart	Domesticated strain
19.	Cataract	<i>Ca</i>	Opaque lens visible in unpigmented eyes, pink-eyed or albino	Cornell Univ., 1943	Smith and Barrentine	Domesticated strain
20.	Jaundice	<i>j</i>	Skin and hair yellow at birth and later	Univ. of Toronto, 1938	C. H. Gunn	Domesticated strain
21.	Shaggy	<i>Sh</i> II	Hair and vibrissae curved, closely linked to curly	Wistar Institute, 1946	Helen Dean King	Domesticated strain

(Table continued on following page)



22.	Silver	<i>s</i>	Black coat interspersed with white hairs	Wistar Institute, 1939	Helen Dean King	Domesticated strain
23.	Fawn	<i>f</i>	Dilutes black to tawny, blue to fawn	Wistar Institute, 1946	Helen Dean King	Domesticated strain

cessive in one animal of the foundation wild stock. Three previously unknown mutations of the rat made their appearance in the captive stock, curly in generation 17, brown in generation 22, and stub seven generations later.

Castle had reported in 1907 the occurrence in wild rats in England of mutations *p* and *r*. One of these, *r*, made its appearance independently in Wistar Institute stock (not captive gray) as reported by King, 1923.

Meanwhile several other mutations had been observed in domestic laboratory stocks. Roberts in 1924 reported the occurrence of hairless (*hr*), and in 1929 of blue dilutin (*d*). Wilder (1932) observed an independent occurrence of the hairless mutation, and Feldman demonstrated the identity of the two. Gregory and Blunn in 1935 reported the occurrence of a second dominant curly mutation which they designated Curly<sub>2</sub>, and showed to be distinct from Curly, the two being independent in inheritance, and so obviously borne in different chromosome pairs. In the same year (1935) Feldman discovered a recessive form of curly hair which he named kinky (*k*).

In 1936 King reported the discovery of a recessive gene for waltzing (*w*) in Wistar albino rats, mutants from captive grays. In (1937) Daniels discovered a recessive gene for wobbly, which was described and its linkage relations canvassed by Castle, King, and Daniels. In 1938 Gunn described a recessive gene for jaundice. In 1939 Smith and Bogart reported the discovery of a recessive lethal, anemia (*an*); and Grüneberg reported on a different lethal (*l*) resulting in an abnormal skeleton. This he showed to be carried in the albino chromosome. In 1941 Greep reported the discovery of incisorlers (*in*). In 1943 Smith and Barrentine reported the discovery of a new dominant mutation cataract (*Ca*). King has also discovered three other mutations on which as yet no publication has been made. They are silver, a recessive; fawn, also recessive; and shaggy, a dominant resembling the curly mutations.

Burhoe, studying the blood groups of the rat, has demonstrated the existence of two dominant agglutininogen genes, *Ag* and *M*.

One striking fact concerning the mutations of the rat is that they may occur again independently of an original and earlier occurrence. King has demonstrated this in her own studies for *c*, *a*, *h* and *r*, also for *Cu*<sub>1</sub> observed

as occurring independently in New Haven, Conn., by Whitney. The blue mutation (*d*) originally observed by Roberts in 1929 was shown to have occurred independently later in New York (Curtis and Dunning, 1940). Curly, as well as Curly<sub>2</sub>, has made a second independent appearance, at Madison, Wis., (personal communication from Dr. A. B. Chapman).

Linkage studies made to discover what genes are carried in a common chromosome pair have been made by Roberts and Quisenberry (1936), by Burhoe and by Feldman, but all their findings were negative. The first positive finding was made by Castle and Wright, who showed that the two genes for yellow coat, *p* and *r* are linked with each other. Later it was found by Castle, Dunn and Wachter that they lie in the same chromosome pair as the albino gene. Castle and King found that the gene for waltzing also lies in the albino chromosome, and Grüneberg added a fifth gene, a lethal *l* to this first linkage group. A second linkage group was found by King and Castle to include curly and brown, to which later were added the genes shaggy, anemia and incisorless. A third linkage group was found by Castle, King and Daniels to include the genes wobbly and hairless. A fourth group includes genes kinky and stub, as demonstrated by Castle and King. Conventional linkage maps may be expressed as follows:

I	<i>p</i>	<i>r</i>	<i>c</i>	<i>l</i>	<i>w</i>
	0	20.5	21	24.3	66.3
II	<i>Cu</i>	<i>Sh</i>	<i>an</i>	<i>in</i>	<i>b</i>
	0	0.5	10.3	24	45
III	<i>wo</i>				<i>hr</i>
	0				40.3
IV	<i>k</i>				<i>st</i>
	0				34.1

For the following genes no linkage has as yet been found, though the investigation is far from complete: *a*, *Ag*, *Ca*, *Cu*<sub>2</sub>, *d*, *h*, *j* and *M*. If all of these should be shown to be independent of the established linkage groups we should have genetic markers for 12 of the 20 autosomal chromosome pairs, no sex-linked gene having as yet been discovered.

By way of summary we may say that the earliest attempts at domestication of the Norway rat followed the discovery in wild populations of conspicuous mutants, albinos, non-agouti blacks and piebalds. These were captured, and intercrossed, resulting in the formation of a race of albinos homozygous for the three mutant genes *c*, *a* and *h*. Such is the genetic constitution of the ordinary laboratory white rat.

Unconscious selection was probably made of the more gentle and tamable individuals for propagation, which also favored increased productiveness in captivity.

Experimental re-enactment of domestication by taking wild gray rats into captivity has resulted in (1) increased body size, (2) decreased savageness (inclination to bite and attempt to escape) and (3) increased fertility.

These may be regarded as consequences of mutations affecting behavior either directly or by way of endocrine changes, rather than as direct effects of a changed environment. At the same time mutations have been observed to occur which affect the structure of the hair or its pigmentation, or the central nervous system (waltzing, wobbly), the eyes or the skeleton (stub tail, lethal *l*). These are not to be regarded as consequences of domestication but purely as sports, spontaneous and without assignable causation.

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$A_g$  is used as the symbol of a gene for agglutinogene A of Burhoe (published here-with) to avoid confusion with  $A$ , agouti, dominant allele of  $a$ .

## AN EXPRESSION OF HOPF'S INVARIANT\* AS AN INTEGRAL

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1. Let  $S^2 \subset R^3$  and  $S^3 \subset R^4$  be spheres in the sense of Euclidean geometry,  $S^2$  having unit radius, and let  $f: S^3 \rightarrow S^2$  be a twice differentiable map. Let  $x^1, x^2, x^3$  be local coördinates for  $S^3$ , let  $\lambda, \mu$  be local coördinates for  $S^2$  and let  $\sigma(\lambda, \mu)$  be the area density on  $S^2$ . Let

$$u_{ij} = \frac{1}{4\pi} \sigma(\lambda, \mu) \frac{\partial(\lambda, \mu)}{\partial(x^i, x^j)} \quad (i, j = 1, 2, 3), \quad (1.1)$$

where  $\lambda, \mu$ , in (1.1) stand for the functions  $\lambda(x^1, x^2, x^3), \mu(x^1, x^2, x^3)$ , by means of which  $f$  is expressed locally. Then  $u_{ij}$  are the components of an alternating tensor in  $S^3$ . It may be verified that the divergence of this tensor vanishes. That is to say

$$\partial u_{23} / \partial x^1 + \partial u_{31} / \partial x^2 + \partial u_{12} / \partial x^3 = 0.$$

Hence, by de Rham's theorem<sup>1</sup> there is a covariant vector-field  $(v_1, v_2, v_3)$ , defined over the whole of  $S^3$ , such that

$$\partial v_i / \partial x^j - \partial v_j / \partial x^i = u_{ij}. \quad (1.2)$$

The main object of this note is to prove that

$$\frac{1}{4\pi} \int \int \int_{S^3} \sigma \begin{vmatrix} v_1 & v_2 & v_3 \\ \frac{\partial \lambda}{\partial x^1} & \frac{\partial \lambda}{\partial x^2} & \frac{\partial \lambda}{\partial x^3} \\ \frac{\partial \mu}{\partial x^1} & \frac{\partial \mu}{\partial x^2} & \frac{\partial \mu}{\partial x^3} \end{vmatrix} dx^1 dx^2 dx^3 = \gamma, \quad (1.3)$$

where  $\gamma$  is the Hopf invariant of the map  $f$ . Notice that this integral can also be expressed in the form

$$\int_{S^3} \omega_1 \square \omega_2 = \gamma, \quad (1.4)$$

where  $\omega_1$  and  $\omega_2$  are the forms whose coefficients are  $v_i$  and  $u_{ij}$ , and  $\square$  denotes Grassmann multiplication.

Before starting the proof we re-state the theorem in topological terms. Let  $w^2$  be the basic co-cycle on  $S^2$ , defined as a function of singular 2-simplexes, and let  $u^2 = f_* w^2$ , where  $f_*$  stands for the map of co-chains, which is dual to  $f$ . Since  $\delta u^2 = 0$ , because  $\delta w^2 = 0$ , and since the second co-homology group of  $S^3$  is trivial, there is a co-chain,  $v^1$ , such that  $\delta v^1 = u^2$ . Our theorem is equivalent to the statement

$$v^1 \smile u^2 = \gamma c^3,$$

where  $c^3$  is the basic co-cycle on  $S^3$ . This form of the result has been obtained independently by N. Steenrod, as an application of a process, which he has developed but has not yet published.

2. We shall prove (1.3) with the help of a theorem concerning 3-dimensional manifolds, which are fibred in the original sense of H. Seifert.<sup>2</sup> Let  $M^3$  be such a manifold, which we assume to be orientable and closed. Let  $f: M^3 \rightarrow M^2$  be a fibre mapping of  $M^3$  on an orientable, 2-dimensional manifold  $M^2$ . Let  $M^3, M^2$ , the map  $f$  and the fibres be twice differentiable. Let each fibre be oriented so as to have a positive intersection with a transverse 2-cell, which takes its orientation from a given orientation of  $M^2$ . We recall Seifert's definition of a *fibre neighborhood*,  $T$ , of a given fibre  $H$  (p. 150), and the number  $n$ , which is the degree of the map  $f|E^2$  in the point  $f(H)$ , where  $E^2$  is a cross section of  $T$ . We shall call  $n$  the *order* of the fibre  $H$ , and  $H$  will be described as a *simple* or a *multiple* fibre, according as  $n = 1$  or  $n > 1$ . In either case we may take  $T = f^{-1}(E_1^2)$ , where  $E_1^2 \subset M^2$  is any sufficiently small 2-element, of which  $f(H)$  is an inner point.

Let  $x^1, x^2, x^3$  be local coördinates for  $M^3$ , let  $\lambda, \mu$  be local coördinates for  $M^2$  and let  $\sigma(\lambda, \mu)$  be an area density for  $M^2$ , which may be given abstractly or defined in terms of a Riemannian metric. Let  $\alpha$  be the reciprocal of the total area of  $M^2$ , which is compact since  $M^3$  is compact, and

let  $u_i$  be defined by (1.1), with  $1/4\pi$  replaced by  $\alpha$ . We assume the existence of a covariant vector-field  $v_i$ , defined over the whole of  $M^3$  and satisfying (1.2). As in Section 1 we write, using the summation convention,

$$(a) \quad \omega_2 = \alpha \sigma(\lambda, \mu)(d\lambda d\mu - \delta\lambda d\mu) = u_i dx^i \delta x^i, \quad (2.1)$$

$$(b) \quad \omega_1 = v_i dx^i.$$

I say that

$$\int_H \omega_1 = \int_{H'} \omega_1 = \gamma, \text{ say,} \quad (2.2)$$

where  $H$  and  $H'$  are any two simple fibres and the integrals are both taken in the positive sense and  $\gamma$  is thus defined. For  $d\lambda = d\mu = 0$  along a fibre whence  $\int \omega_2 = 0$  over any surface which is generated by fibres. Let  $\Delta \subset M^2$  be a smooth, non-singular arc joining  $f(H)$  to  $f(H')$  and not containing the image of any multiple fibre. Then  $\Sigma = f^{-1}(\Delta)$  is generated by fibres and is obviously bounded by  $\pm(H - H')$ . Therefore

$$\int_H \omega_1 - \int_{H'} \omega_1 = \pm \int_{\Sigma} \omega_2 = 0,$$

which establishes (2.2).

3. Let  $D$  denote the determinant in (1.3). The theorem referred to in Section 2 is that

$$\alpha \int \int \int \sigma D dx^1 dx^2 dx^3 = \gamma, \quad (3.1)$$

where  $\gamma$  is defined by (2.2). Let  $H$  be a simple fibre and  $T$  a fibre neighborhood of  $H$ . We may represent  $T$  as the topological product  $E^2 \times H$  where  $E^2$  is a cross-section of  $T$ , which is mapped topologically on  $f(E^2)$ , with non-degenerate Jacobian. We assume that  $f(E^2)$  is contained in the domain of a local coördinate system,  $(\lambda, \mu)$ , for  $M^2$ , and use the same coördinates for  $p \in E^2$  as for  $f(p)$ . Thus  $T$  may be referred to coördinates  $(\lambda, \mu, \theta)$ , such that  $f(\lambda, \mu, \theta) = (\lambda, \mu)$  and  $\theta \in < 0, 2\pi >$  is a periodic coördinate for  $H$ . Let  $\omega_1 = w_\lambda d\lambda + w_\mu d\mu + w_\theta d\theta$  and let  $H(\lambda, \mu) = f^{-1}(\lambda, \mu)$ . Then  $D = w_\theta(\lambda, \mu, \theta)$  and

$$\gamma = \int_{H(\lambda, \mu)} \omega_1 = \int_0^{2\pi} w_\theta(\lambda, \mu, \theta) d\theta.$$

Hence the integral in (3.1), extended over  $T$ , becomes

$$\begin{aligned} \alpha \int \int \int \sigma(\lambda, \mu) w_\theta(\lambda, \mu, \theta) d\lambda d\mu d\theta \\ &= \alpha \int \int_{E^2} \left\{ \sigma(\lambda, \mu) \int_0^{2\pi} w_\theta(\lambda, \mu, \theta) d\theta \right\} d\lambda d\mu \\ &= \alpha \gamma \int \int_{E^2} \sigma(\lambda, \mu) d\lambda d\mu \\ &= \alpha \gamma \times \text{area of } f(E^2). \end{aligned} \quad (3.2)$$



There are but a finite number of singular fibres in  $M^3$ . Let  $q_1, \dots, q_m \subset M^2$  be their images under  $f$  and let  $K^2$  be a triangulation of  $M^2 - (q_1 \cup \dots \cup q_m)$ , which will be an infinite complex if there are multiple fibres. Let the simplexes in  $K^2$  be so small that  $T = f^{-1}(\tau^2)$  is a fibre neighborhood of  $H = f^{-1}(q)$ , for each 2-simplex  $\tau^2$  in  $K^2$  and any inner point  $q \in \tau^2$ . We may also suppose that each  $\tau^2$  is in the domain of a local coördinate system for  $M^2$ . Then (3.1) is established by summing the equalities (3.2), with  $T = f^{-1}(\tau^2)$ , for each  $\tau^2$  in  $K^2$ .

4. Now let a simple fibre,  $H$ , bound<sup>4</sup> a surface,  $C$ , which we assume to be piecewise differentiable. Let  $C$  be given locally by  $x^i = x^i(\xi, \eta)$ , where  $x^i(\xi, \eta)$  are differentiable functions of the parameters  $\xi, \eta$ . Then, by (1.2), (1.1) and Stokes' Theorem we have

$$\begin{aligned} \gamma = \int_H v_i dx^i &= \alpha \int_C \int \sigma \left\{ \frac{\partial(\lambda, \mu)}{\partial(x^2, x^3)} \frac{\partial(x^2, x^3)}{\partial(\xi, \eta)} + \frac{\partial(\lambda, \mu)}{\partial(x^3, x^1)} \times \right. \\ &\quad \left. \frac{\partial(x^3, x^1)}{\partial(\xi, \eta)} + \frac{\partial(\lambda, \mu)}{\partial(x^1, x^2)} \frac{\partial(x^1, x^2)}{\partial(\xi, \eta)} \right\} d\xi d\eta \\ &= \alpha \int_C \int \sigma \frac{\partial(\lambda, \mu)}{\partial(\xi, \eta)} d\xi d\eta, \end{aligned}$$

which is the degree of the map  $f|C$ . Hence  $\gamma$  is the Hopf invariant of the map  $f$ , and (1.3) is established in case  $f: S^3 \rightarrow S^2$  is a fibre mapping.

5. Let  $S^2$  be given by  $x^2 + y^2 + z^2 = 1$  and  $S^3$  by  $|\xi|^2 + |\eta|^2 = 1$ , where  $x, y, z$  are Cartesian coördinates for  $R^3$  and  $\xi, \eta$  are complex coördinates for  $R^4$ . Let  $f: S^3 \rightarrow S^2$  be given by

$$x + iy = 2\rho\xi^m\bar{\eta}^n, \quad z = \rho(|\xi|^{2m} - |\eta|^{2n}),$$

where  $\rho = 1/(|\xi|^{2m} + |\eta|^{2n})$  and  $m, n$  are any (positive, zero or negative) integers, which are prime to each other (unless one or both is zero). Then,  $f$  is a fibre map, with fibres given by<sup>5</sup>  $\xi = \xi_0 e^{in\theta}$ ,  $\eta = \eta_0 e^{im\theta}$ , and its Hopf invariant<sup>6</sup> is  $\pm mn$ , the sign depending on the orientation of  $S^3$ . Thus  $m$  and  $n$  may be chosen, say with  $m = \pm\gamma$ ,  $n = 1$ , so that  $f$  has a given invariant, and is therefore homotopic<sup>7</sup> to a given map  $S^3 \rightarrow S^2$ .

6. We complete the proof of (1.3) by showing that the integral is an invariant of the homotopy class of the map  $f$ . This will be included in a more general result, which has no reference to fibre maps. Let  $M^3$  and  $M^2$  be twice differentiable manifolds and  $f: M^3 \rightarrow M^2$  any twice differentiable map. Let  $\omega_2$  be the form in  $M^2$  which is given by (2.1), and assume that  $\omega_2$  is an exterior derivative. Let  $\omega_1' = \omega_2$ , where  $\omega'$  denotes the exterior derivative of  $\omega$ . Then we prove that

(A) The integral

$$I(f) = \int_{M^3} \omega_1 \square \omega_2$$

is independent of the choice of the form  $\omega_1$ , satisfying  $\omega_1' = \omega_2$ .

(B) Let  $F: M^3 \rightarrow M^2$  be any twice differentiable map, which is homotopic to  $f$ . Then the form  $\Omega_2$ , given by (2.1) in terms of  $F$ , is also an exterior derivative, and

(C)  $I(F) = I(f)$ .

The statement (A) follows at once from the fact that, if  $\omega_1' = \bar{\omega}_1' = \omega_2$ , then  $\bar{\omega}_1 - \omega_1$  is a closed form. Since  $\omega_2$  is an exterior derivative, so is  $(\bar{\omega}_1 - \omega_1) \square \omega_2$ , whence

$$\int_{M^3} \bar{\omega}_1 \square \omega_2 - \int_{M^3} \omega_1 \square \omega_2 = \int_{M^3} (\bar{\omega}_1 - \omega_1) \square \omega_2 = 0.$$

In order to prove (B) and (C) we imbed  $M^2$  as an analytic surface in  $R^3$ . Let  $N$  be the open set consisting of points whose distance from  $M^2$  is less than a positive  $\rho_1$ , which is so small that no two normals to  $M^2$  intersect in  $N$ . Let  $\rho > 0$  be so small that, if  $\delta(q, q') \leq \rho$ , where  $q, q' \in M^2$  and  $\delta(q, q')$  is the Euclidean distance from  $q$  to  $q'$ , then the linear segment, which is given in vector notation by  $(1-t)q + tq'$ , for  $-1 \leq t \leq 2$ , lies in  $N$ . This being so, let  $\phi(q, q', t)$  be the normal projection of  $(1-t)q + tq'$  on  $M^2$ .

It is clearly sufficient to prove (B) and (C) in case  $\delta\{f(p), F(p)\} \leq \rho$  for each  $p \in M^3$ . Let this be so and let  $M^4$  be the open manifold  $M^3 \times (-1, 2)$ . Then a twice-differentiable map,  $g: M^4 \rightarrow M^2$ , is defined by

$$g(p \times t) = \phi\{f(p), F(p), t\},$$

and  $g(p \times 0) = f(p)$ ,  $g(p \times 1) = F(p)$ . Let  $x^1, x^2, x^3$  be local coördinates for  $M^3$  and let the map  $g$  be given locally by  $\lambda = \lambda(x^1, x^2, x^3, t)$ ,  $\mu = \mu(x^1, x^2, x^3, t)$ . Let  $\bar{\omega}_2$  be the form in  $M^4$ , which is given by (2.1). Any cycle in  $M^4$  is homologous to a cycle in  $M^3 \times 0$ , on which  $\bar{\omega}_2$  reduces to the form,  $\omega_2(0)$ , associated with the map  $f$ . Since the latter is an exterior derivative its integral over any cycle in  $M^3$  is zero. Therefore the integral of  $\bar{\omega}_2$  over any cycle in  $M^4$  is zero.

Let  $M_0^4 = M^3 \times \langle 0, 1 \rangle$ , so that  $M_0^4$  is a bounded manifold in  $M^4$ . On p. 66, *et seq.*, of de Rham's paper let  $A$  be a complex covering  $M_0^4$ . Then, with other minor adjustments in his arguments, it follows that there is a form  $\bar{\omega}_1$ , defined throughout an open set,  $U \subset M^4$ , which contains  $M_0^4$ , such that  $\bar{\omega}_1' = \bar{\omega}_2$  in  $U$ . Since  $M_0^4$  is compact we may take  $U = M^3 \times (-\epsilon, 1 + \epsilon)$  for some  $\epsilon > 0$ .

Since  $\bar{\omega}_1' = \bar{\omega}_2$  in  $U$  we have  $\omega_1'(t) = \omega_2(t)$  ( $-\epsilon < t < 1 + \epsilon$ ), where  $\omega_1(t)$  and  $\omega_2(t)$  are the forms which are obtained from  $\bar{\omega}_1$  and  $\bar{\omega}_2$  by writing  $dt = 0$ . This establishes (B), since  $\omega_2(0) = \omega_2$ ,  $\omega_2(1) = \Omega_2$ , regarding  $\omega_1(t)$  and  $\omega_2(t)$  as forms in  $M^3$ , which depend on the parameter  $t$ .

In order to prove (C) it is sufficient to prove that  $\partial\omega_3(t)/\partial t$  is an exterior derivative, where  $\omega_3(t) = \omega_1(t) \square \omega_2(t)$  ( $-\epsilon < t < 1 + \epsilon$ ). For, treating  $\omega_1(t)$ ,  $\omega_2(t)$ ,  $\omega_3(t)$  as forms in  $M^3$ , which depend on the parameter  $t$ , we have

$$\frac{d}{dt} \int_{M^1} \omega_1(t) \square \omega_2(t) = \int_{M^1} \partial \omega_3(t) / \partial t = 0$$

if  $\partial \omega_3(t) / \partial t$  is an exterior derivative. To prove that it is we write  $\bar{\omega}_3 = \bar{\omega}_1 \square \bar{\omega}_2$ . Then  $\bar{\omega}_3' = \bar{\omega}_1' \square \bar{\omega}_2 = \bar{\omega}_2 \square \bar{\omega}_1'$ , and it is clear from (2.1) that  $\bar{\omega}_2 \square \bar{\omega}_1' = 0$ . Let  $u_{abc}$  ( $a, b, c = 1, \dots, 4; x^4 = t$ ) be the coefficients of  $\bar{\omega}_3$ . Since  $\bar{\omega}_3' = 0$  we have

$$\frac{\partial u_{jk4}}{\partial x^i} - \frac{\partial u_{ik4}}{\partial x^j} + \frac{\partial u_{ij4}}{\partial x^k} - \frac{\partial u_{ijk}}{\partial t} = 0 \quad (i, j, k = 1, 2, 3).$$

But  $u_{ijk}$  ( $i, j, k = 1, 2, 3$ ) are the coefficients of  $\omega(t)$ . Therefore  $\partial \omega_3(t) / \partial t = \theta_2'$ , where  $\theta_2 = u_{ij4} dx^i dx^j$ , and the proof is complete.

7. We conclude with some additional observations. First notice that (3.2) is valid even if  $H$  is a fibre of order  $n > 1$ . For, provided the total area of  $M^2$  is finite, (3.1) is true for open as well as for closed manifolds, the proof being the same in each case. Therefore (3.2), with  $n > 1$ , follows from (3.1), applied to the interior of  $T$ .

Also

$$\gamma = n \int_H \omega_1 \quad (7.1)$$

if  $H$  is a fibre of order  $n$ . For if  $H'$  is a simple fibre in a fibre neighborhood of  $H$  it is obvious from the definition of a fibre neighborhood that there is a surface which is generated by fibres and bounded by  $\pm(H' - nH)$ . Therefore (7.1) is proved in the same way as (2.2).

Also if  $H$  is a fibre of order  $n$  and  $E^2$  is a cross-section of a fibre neighborhood,  $T$ , of  $H$ , then  $f$  maps  $E^2$  on  $f(E^2)$  with degree  $n$ . Combining these three results, we have

$$\begin{aligned} \int_T \omega_1 \square \omega_2 &= \alpha \gamma \times \text{area of } f(E^2) \\ &= \alpha n \int_H \omega_1 \times \frac{1}{n} \int_{E^2} \sigma(\lambda, \mu) d\lambda d\mu \\ &= \int_H \omega_1 \times \int_{E^2} \omega_2 \\ &= \int_H \omega_1 \times \int_m \omega_1, \end{aligned} \quad (7.2)$$

where  $m$  is the boundary of  $E^2$ .

8. Let us now start with  $\omega_1$ , that is to say with a covariant vector-field  $V$ , which is defined all over  $M^3$ . Then  $\text{curl } V$  is an alternating covariant tensor, which is the same as a contravariant vector density. Therefore "lines of flow" or "lines of force" are defined by  $\text{curl } V$  and we assume them to be the fibres discussed in Section 2. Let  $H$  be a given

fibre and  $T$  a fibre neighborhood of  $H$ . Let  $\lambda, \mu, \theta$  be the coördinates for  $T$ , which were defined in Section 3, except that we restrict  $\theta$  to the interval  $-1/n < \theta < 1/n$ . Then these coördinates are valid even if  $H$  is a multiple fibre, of order  $n$ . In these coördinates  $\text{curl } V$  has components of the form  $0, 0, u(\lambda, \mu, \theta)$ , and

$$\frac{\partial u}{\partial \theta} = \text{div}(\text{curl } V) = 0.$$

From the transformation law of the components,  $u_{ij}$ , of  $\text{curl } V$ , namely

$$V_{ab} = u_{ij} \frac{\partial x^i}{\partial y^a} \frac{\partial x^j}{\partial y^b},$$

it follows that  $\sigma(\lambda, \mu) = u(\lambda, \mu, \theta)$  is unaltered by coördinate transformations of the form  $\theta' = \theta'(\lambda, \mu, \theta)$ ,  $\lambda' = \lambda$ ,  $\mu' = \mu$  and transforms in the same way as a scalar density in  $M^2$  under transformations of the form  $\lambda' = \lambda'(\lambda, \mu)$ ,  $\mu' = \mu'(\lambda, \mu)$ ,  $\theta' = \theta$ . Hence  $\text{curl } V$ , whose components in the coördinates  $(\lambda, \mu, \theta)$  are  $0, 0, \sigma(\lambda, \mu)$ , is related to the fibre mapping  $(\lambda, \mu, \theta) \rightarrow (\lambda, \mu)$  by the equations (1.1), with the factor  $1/4\pi$  discarded. In the notation of vector calculus (7.2) becomes

$$\int_T \int \int V \cdot \text{curl } V \, d\tau = \int_H V \cdot ds \times \int_M V \cdot ds$$

\* Hopf, H., *Math. Annalen*, **104**, 637-665 (1931).

<sup>1</sup> de Rham, G., *Journal de Math p. et a.*, **96**, 185 (1931).

<sup>2</sup> *Acta Mathematica*, **60**, 147-238 (1933).

<sup>3</sup>  $\sigma > 0$  in positively oriented coördinate systems.

<sup>4</sup> In any case the fact that  $\omega_2$  is an exterior derivative implies that  $H$  bounds with division.

<sup>5</sup> Cf. Seifert, *loc. cit.*, p. 159.

<sup>6</sup> Let  $H_1, H_2$  be fibres of orders  $m, n$ . Then  $H \sim mH_1$  in  $S^3 - H_2$  and  $H' \sim nH_2$  in  $S^3 - H$ , where  $H, H'$  are simple fibres near  $H_1, H_2$ , respectively, and it follows that  $\gamma = \pm mn L(H_1, H_2)$ , where  $L$  denotes the linking coefficient. In this case  $H_1$ , given by  $\eta = 0$ , is of order  $n$  and  $H_2$ , given by  $\xi = 0$ , is of order  $m$ , and  $L(H_1, H_2) = \pm 1$  (cf. Hopf, *loc. cit.*, p. 655).

<sup>7</sup> Hurewicz, W., *Proc. Akad. Amsterdam*, **38**, 119 (1935). See also H. Freudenthal, *Compositio Math.*, **5**, 299-314 (1937).

## COHOMOLOGY INVARIANTS OF MAPPINGS

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The Hopf invariant<sup>1</sup> of a map of a  $(2n - 1)$ -manifold on an  $n$ -manifold, and the Gysin<sup>2</sup> extension of the Hopf mechanism appear as special cases of the following general operation involving a map, cocycles and their products.

Let  $K, K'$  be complexes, and  $f: K \rightarrow K'$  a simplicial map. Then  $f$  induces homomorphisms of the cochain groups and cohomology groups with integer coefficients. These are denoted by

$$f': C^p(K') \rightarrow C^p(K), \quad f^*: H^p(K') \rightarrow H^p(K).$$

Suppose  $u \in H^p(K'), v \in H^q(K')$  are such that

$$f^*u = 0, \quad u \smile v = 0. \quad (1)$$

Choose representatives cocycles  $u', v'$  of  $u, v$ . By (1), there exist cochains  $a \in C^{p-1}(K), b \in C^{p+q-1}(K')$  such that

$$f'u' = \delta a, \quad u' \smile v' = \delta b.$$

It follows that

$$a \smile f'v' - f'b \quad (2)$$

is a  $(p + q - 1)$ -cocycle of  $K$ . A different choice of  $u', v', a, b$  usually alters the cohomology class of (2). However, it does so by an element of the subgroup of  $H^{p+q-1}(K)$  generated by the two subgroups  $f^*H^{p+q-1}(K')$  and  $H^{p-1}(K) \smile f^*v$ . Hence (2) defines a unique element

$$[f, u, v] \in H^{p+q-1}(K) / [f^*H^{p+q-1}(K') + H^{p-1}(K) \smile f^*v]. \quad (3)$$

In particular, if  $f: S^3 \rightarrow S^2$  is a map of a 3-sphere on a 2-sphere, then any  $u, v \in H^2(S^2)$  satisfy (1), and  $[f, u, v] \in H^3(S^3)$ . In case  $u = v$  is a generator of  $H^2(S^2)$ , it can be proved that

$$[f, u, u] = \gamma z \quad (4)$$

where  $z$  generates  $H^3(S^3)$  and the integer  $\gamma$  is the Hopf invariant of  $f$ .

This last result has a direct interpretation in tensor form by means of the de Rham theorem as formulated by Whitney.<sup>3</sup> Let  $U$  be a covariant second order, alternating tensor field over  $S^2$  whose integral over  $S^2$  is 1. Assume  $f$  to be differentiable, and let  $f'U$  denote the field induced in  $S^3$  by  $U$  and  $f$ . Since the outer derivative of  $U$  is 0, so also is that of  $f'U$ . Since the second Betti number of  $S^3$  is 0, it follows from de Rham's theorem

that there exists a vector field  $A$  over  $S^3$  whose outer derivative is  $f'U$ . According to Whitney, the outer product  $A \cdot f'U$  corresponds to the cup product  $a \smile f'u$ . Therefore, by (4), the integral  $A \cdot f'U$  over  $S^3$  is  $\gamma$ . This form of the result was obtained independently by J. H. C. Whitehead<sup>4</sup> who called my attention to the connection.

The principal property of the operation (3) is

1°. If  $f$  is homotopic of  $g$ , then  $[f, u, v] = [g, u, v]$ .

This permits defining (3) for any continuous  $f$  by the method of simplicial approximation.

2°.  $[f, u, v]$  is linear in  $u$ . For  $v$ 's satisfying (1) and  $f^*v = 0$ , it is linear in  $v$  and  $[f, u, v] = (-1)^{pq}[f, v, u]$ .

3°. If  $f: K \rightarrow K'$ ,  $g: K' \rightarrow K''$  and  $u, v$  on  $K''$  satisfy (1) with  $g, K''$  in place of  $f, K'$ , then  $u, v$  satisfy (1) with  $gf, K''$  in place of  $f, K'$  and

$$f^*[g, u, v] = [gf, u, v].$$

The left side is defined by choosing a representative of  $[g, u, v]$  in  $H^{p+q-1}(K')$ , forming its  $f^*$  image and reducing by the appropriate subgroup.

4°. If  $f: K \rightarrow K'$ ,  $g: K' \rightarrow K''$  and  $u, v$  on  $K''$  satisfy (1) with  $gf, K''$  in place of  $f, K'$ , then  $g^*u, g^*v$  satisfy (1) and

$$[f, g^*u, g^*v] = [gf, u, v] \pmod{[f^*H^{p+q-1}(K') + H^{p-1}(K) \smile f^*v]}.$$

5°. If  $w \in H'(K')$  and  $u, v$  satisfy (1), then both  $w \smile u, v$  and  $u, v \smile w$  satisfy (1) and

$$f^*w \smile [f, u, v] = (-1)^r [f, w \smile u, v], \quad [f, u, v] \smile f^*w = [f, u, v \smile w].$$

The products on the right sides are defined by choosing representatives in the cohomology groups of  $K$ , multiplying, and then reducing by the appropriate subgroup.

The novel feature of the operation (3) is its use of those parts of the cohomology groups on which  $f^*$  is trivial, namely: the kernel of  $f^*$  and the factor group of  $H^{p+q-1}(K)$  by the image of  $f^*$ . The operation is potentially richest in the case of just those maps heretofore called "algebraically inessential." It would seem appropriate to narrow the meaning of algebraically inessential to include only those maps  $f$  for which  $f^* = 0$  and each  $[f, u, v] = 0$ .

Several examples will indicate the ability of the operation to distinguish between maps of the same homology type.

A. Let  $Y$  be composed of two circles  $\alpha, \beta$  with a common point. Let  $X$  be a circle, and let  $f$  map  $X$  into the commutator  $\alpha\beta\alpha^{-1}\beta^{-1}$ . Then  $f$  is homologically trivial. However, if  $u, v$  are generating 1-cocycles on  $\alpha, \beta$ , then  $[f, u, v]$  generates  $H^1(X)$ .

B. Let  $Y = S^2 \cup S^1$  be the union of a 2-sphere and a 1-sphere with a common point  $y_0$ . Let  $X$  be a 2-sphere represented as a long tube  $T$  with



two caps  $C_1, C_2$ . Orient  $C_1, C_2$  concordantly and map each on  $S^2$  with degrees  $+1, -1$ , respectively, and so that their boundaries are mapped on  $\mathfrak{y}_0$ . Map  $T$  once around  $S^1$ . Then  $f$  is homologically trivial. However, if  $u$  is a generating 2-cocycle on  $S^2$ , and  $v$  a generating 1-cocycle on  $S^1$ , then  $[f, u, v]$  generates  $H^2(X)$ .

C. Let  $Y$  be projective 3-space, and  $f: S^3 \rightarrow Y$  the 2-fold covering. Then  $H^2(Y)$  has one non-zero element  $u$ , and  $[f, u, u]$  is the non-zero element of  $H^3(S^3)/f^*H^3(Y)$ .

D. Let  $M, N$  be manifolds of dimensions  $p, q$  and let  $u, v$  be generating  $p, q$ -cocycles. Form  $M \times N$ . Let  $m_0 \in M, n_0 \in N$ , and let  $Y = M \times n_0 \cup m_0 \times N$ . Let  $U$  be a closed normal neighborhood of  $Y$  in  $M \times N$ , and let  $F$  be the retraction of  $U$  into  $Y$ . Let  $X$  be the boundary of  $U$  and  $f = F|X$ . Then  $X$  is a  $(p + q - 1)$ -manifold and  $[f, u, v]$  is its generating  $(p + q - 1)$ -cocycle. (Note that examples  $A, B$  are special cases of  $D$ .)

E. If, in  $D$ ,  $M$  and  $N$  are spheres, then  $X$  is a sphere, and  $f$  represents the J. H. C. Whitehead product  $[M, N]$ .

The operation can also be defined for relative cohomology groups  $H^p(K, L)$  (based on cocycles of  $K$  which are 0 on  $L$ ). Let  $L_1, L_2$  be subcomplexes of  $K$  and  $L_3 = L_1 \cup L_2$ ; and similarly  $K', L'_1, L'_2, L'_3$ . Suppose  $f: (K, L_1, L_2) \rightarrow (K', L'_1, L'_2)$ . Then  $f$  induces homomorphisms

$$f_i^*: H^p(K', L'_i) \rightarrow H^p(K, L_i) \quad i = 1, 2, 3.$$

Suppose  $u \in H^p(K', L'_1), v \in H^q(K', L'_2), f_1^*u = 0, u \smile v = 0$  in  $H^{p+q}(K', L'_3)$ . Then (2) defines a product

$$[f, u, v] \in H^{p+q-1}(K, L_3)/[f^*H^{p+q-1}(K', L'_3) + H^{p-1}(K, L_1) \smile f^*v]. \quad (3')$$

A second extension involves the squaring operations  $u \smile_i u$  introduced by me in a recent paper.<sup>5</sup> If  $u \in H^p(K', L')$ , then  $u \smile_0 u = u \smile u \in H^{2p}(K', L')$  and  $u \smile_i u$  ( $i = 1, 2, \dots$ ) is an element of  $H^{2p-i}(K', L')$  (reduced mod 2 if  $p - i$  is even). Assuming  $f^*u = 0$  in  $H^p(K, L)$  and  $u \smile_i u = 0$ , choose as before  $u', a, b$  and form

$$a \smile_{i-1} a + a \smile_i f'u' - f'b. \quad (4)$$

Then (4) is a  $(2p - i - 1)$ -cocycle of  $K$  which is 0 on  $L$  (mod 2 if  $p - i$  is even). Alteration of the choice of  $u', a, b$  varies the cohomology class of (4) by an element of the subgroup of  $H^{2p-i-1}(K, L)$  spanned by  $f^*H^{2p-i-1}(K', L')$  and squares of order  $i - 1$  of elements of  $H^{p-1}(K, L)$ . Thus (4) defines

$$[f, u, v]_i \in H^{2p-i-1}(K, L)/[f^*H^{2p-i-1}(K', L') + Sq_{i-1}H^{p-1}(K, L)]. \quad (5)$$

(These groups are reduced mod 2 if  $p - i$  is even.)

Now suppose  $u \in H^p(L')$  and

$$\delta: H^p(L') \rightarrow H^{p+1}(K', L')$$

is the homomorphism induced by attaching to each cocycle of  $L$  its co-boundary in  $K - L$ . If  $f^*u = 0$  in  $H^p(L)$ , and  $u \smile u = 0$  in  $H^{2p-1}(L')$ , it follows that  $f^*\delta u = 0$  in  $H^{p+1}(K, L)$ , and  $\delta u \smile_{i+1} \delta u = 0$  in  $H^{2p-1+1}(K', L')$ . Thus

$$[f, u, u]_i \in H^{2p-i-1}(L)/[f^*H^{2p-i-1}(L') + Sq_{i-1}H^{p-1}(L)] \quad (6)$$

$$[f, \delta u, \delta u]_{i+1} \in H^{2p-i}(K, L)/[f^*H^{2p-i}(K', L') + Sq_i H^p(K, L)] \quad (7)$$

are defined. It follows now that

$$\delta[f, u, u]_i = [f, \delta u, \delta u]_{i+1} \quad (8)$$

where the left side is defined by choosing a representative, applying  $\delta$ , and reducing by the subgroup in (7).

If one starts with  $f: S^3 \rightarrow S^2$ , and the result of (4), then applies the operation of *Einhangung* and (8), the following result is obtained: If  $f: S^{n+1} \rightarrow S^n$ , and  $u$  generates  $H^n(S^n)$ , then

$$[f, u, u]_{n-2} = \gamma z \in H^{n+1}(S^{n+1}) \mod 2 \quad (9)$$

where  $z$  is the generator of  $H^{n+1}(S^{n+1})$  and  $\gamma = 0$  if  $f$  is inessential,  $\gamma = 1$  if  $f$  is essential.

Let  $L$  be a subcomplex of  $K$ ,  $f: L \rightarrow S^n$ , and suppose the problem is to determine whether or not  $f$  can be extended to a map  $f': K \rightarrow S^n$ . Choose an  $(n+1)$ -cell  $E$  whose boundary is  $S^n$ . In any case  $f$  extends to a map  $F: (K, L) \rightarrow (E, S^n)$ . Let  $u$  generate  $H^{n+1}(E, S^n)$ . Then  $F^*u \in H^{n+1}(K, L)$  and is precisely the primary obstruction<sup>5</sup> to the extension of  $f$  to  $f'$ . If  $F^*u = 0$ , then  $f$  can be extended to a map of the  $(n+1)$ -skeleton of  $K$  into  $S^n$ . Then a secondary obstruction  $z^{n+2}(f)$  is obtained. We have shown that this is a unique element of

$$H^{n+2}(K, L)/Sq_{n-2}H^n(K, L) \mod 2.$$

Using (8) and (9), it can be proved that

$$z^{n+2}(f) = [F, u, u]_{n-1} \quad n > 2.$$

Thus, if  $\dim(K - L) = n + 2$ , then  $[F, u, u]_{n-1} = 0$  is a necessary and sufficient condition for the existence of the extension  $f'$ . If  $\dim(K - L) > n + 2$ , the vanishing of all  $[F, u, u]_i$  ( $i = 0, 1, \dots, n$ ) is a necessary condition for the existence of  $f$ . With more information concerning the homotopy groups of  $S^n$ , one might hope to prove it sufficient if  $\dim(K - L) < 2n$ .

Proofs of properties of  $[f, u, v]$  based on the cochain definition (2) are long and cumbersome. The following second definition uses only well-established invariant properties of cohomology groups and products so that  $[f, u, v]$  is invariant by definition. Proofs based on this definition are much simpler.

Let  $X, Y$  be topological spaces,  $f$  a map  $X \rightarrow Y$ , and let  $u \in H^p(Y)$ ,  $v \in H^q(Y)$  satisfy (1). Let  $C$  be the mapping cylinder of  $f$ , and  $F : C \rightarrow Y$  its projection. Let

$$i: X \rightarrow C, \quad j: (C, 0) \rightarrow (C, X)$$

be identity maps. Since  $F$  is homotopic to the identity map of  $C$ ,  $F^*$  is isomorphic. Let  $\bar{u} = F^*u$ ,  $\bar{v} = F^*v$ . Then  $\bar{u} \smile \bar{v} = F^*(u \smile v) = 0$ . Furthermore  $i^*\bar{u} = i^*F^*u = (Fi)^*u = f^*u = 0$ . By exactness of the cohomology sequence of  $(C, X)$ , there exists a  $u' \in H^p(C, X)$  such that  $j^*u' = \bar{u}$ . Form  $u' \smile \bar{v} \in H^{p+q}(C, X)$ . Then  $j^*(u' \smile \bar{v}) = j^*u' \smile \bar{v} = \bar{u} \smile \bar{v} = 0$ . By exactness of the cohomology sequence of  $(C, X)$ , there exists an element  $w \in H^{p+q-1}(X)$  such that  $\delta w = u' \smile \bar{v}$ . An examination of the effect of choosing different elements  $u', w$  shows that  $w = -[f, u, v]$  is unique mod  $[f^*H^{p+q-1}(Y) + H^{p-1}(X) \smile f^*v]$ .

<sup>1</sup> *Math. Annalen*, 104, 637–665 (1931); *Fund. Math.*, 25, 427–440 (1935).

<sup>2</sup> *Comment. Math. Helv.*, 14, 61–122 (1942).

<sup>3</sup> *Bull. Amer. Math. Soc.*, 43, 785–805 (1937).

<sup>4</sup> See also "An Expression of Hopf's Invariant as an Integral," *Proc. Nat. Acad. Sci.*, 33, 117–123 (1947).

<sup>5</sup> "Products of Cocycles and Extensions of Mappings," to appear in the *Annals of Math.*, No. 2 (1947).

## THE $(L^2)$ -SPACE OF RELATIVE MEASURE

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1. Let a real- or complex-valued function  $f = f(x)$ , where  $0 < x < \infty$ , be called of class  $(N^2)$  if it satisfies the following conditions:  $f(x)$  is of class  $(L^2)$  on every bounded interval  $(0, X)$  and the mean-value,  $M(|f|^2)$ , of  $|f|^2$  exists as a finite limit, where the operator  $M$  is defined by

$$M(g) = \lim_{X \rightarrow \infty} \int_0^X g(x) dx / X. \quad (1)$$

If  $f$  and  $g$  are of class  $(N^2)$ , then  $\bar{M}(|f - g|^2) < \infty$ , where

$$\bar{M}(p) = \limsup_{X \rightarrow \infty} \int_0^X p(x) dx / X, \quad (p \geq 0). \quad (2)$$

Since  $\bar{M}(p_1 + p_2) \leq \bar{M}(p_1) + \bar{M}(p_2)$ , it follows that a metric function space, to be called the  $(N^2)$ -space, can be defined as follows: The elements of the space consist of all functions of class  $(N^2)$ , and two elements,  $f$  and  $g$ , of the space are considered as identical if their distance is zero, with the under-

standing that the distance is defined as the non-negative square root of  $\bar{M}(|f - g|^2)$ .

It is clear that, when  $f(x)$  is periodic (for  $x > 0$ ), it is of class  $(N^2)$  if and only if it is of class  $(L^2)$  over a period, and that the metric of the space  $(N^2)$  then becomes identical with the metric of the corresponding  $(L^2)$ -subspace. More generally, it is clear that, if almost-periodicity  $(B^2)$  is referred to the half-line  $x > 0$ , then  $f(x)$  is of class  $(N^2)$  whenever it is of class  $(B^2)$ , and that the metric of the space  $(N^2)$  becomes identical with the metric of its  $(B^2)$ -subspace. However, while both subspaces  $(L^2)$ ,  $(B^2)$  are linear vector spaces, it turns out that the full space  $(N^2)$  is not linear. On the other hand, the completeness property of the subspaces  $(L^2)$ ,  $(B^2)$  (Fischer-Riesz, Besicovitch) proves to be shared by the full space  $(N^2)$ .

2. Let  $f(x)$  be called of class  $(N^2)_0$  if it is a bounded function of  $x$  and is of class  $(N^2)$ , and let the metric space  $(N^2)_0$  be defined as the function space consisting of the functions of class  $(N^2)_0$  and carrying the metric of the  $(N^2)$ -space.

By considerations rediscovered by Besicovitch (1926), Nalli<sup>1</sup> (1914) proved that, if a sequence of elements in  $(N^2)_0$  is a Cauchy sequence, then it has a limit in  $(N^2)$ . In addition, Nalli<sup>2</sup> emphasized that her result implies an analogue of the Fischer-Riesz theorem for the "orthogonal system"  $e^{i\lambda x}$ ,  $-\infty < \lambda < \infty$ , of the  $(B^2)$ -space.

However, the  $(N^2)_0$ -space is not complete. In order to see this, it is sufficient to consider the sequence of partial sums of the Fourier series of any periodic function of class  $(L^2)$  which fails to be bounded (even if  $x$ -sets of measure zero are disregarded).

3. *The space  $(N^2)$  is not a linear space.*<sup>3</sup>

This is equivalent to the statement that  $f_1$  and  $f_2$  can be in  $(N^2)$  when  $f_1 + f_2$  is not. When  $f_1$  and  $f_2$  are real, this, in turn, is equivalent to the statement that the mean-value, (1), of the product  $g = f_1 f_2$  of two functions of class  $(N^2)$  need not exist. Hence, if  $f_1$  is chosen to be the constant 1 and if  $f_2$  is denoted by  $f$ , it follows that it is sufficient to ascertain the existence of a function  $f$  of class  $(N^2)$  for which  $M(f)$  fails to exist. But such an  $f$  results by choosing  $f(x)$  to be  $+1$  or  $-1$  according as  $x$  is or is not in  $S$ , where  $S$  is a sequence of  $x$ -intervals having the following property: The mean-value  $M(h)$  does not exist if  $h$  is the characteristic function of  $S$  (that is,  $h(x)$  is 1 or 0 according as  $x$  is or is not in  $S$ ). Since the existence of such  $S$  or  $h(x)$  is obvious, it follows that  $(N^2)$  is not linear.

*There does not exist in  $(N^2)$  a linear subspace containing all linear subspaces of  $(N^2)$ .*

This contains the preceding fact (since otherwise the space  $(N^2)$  itself would be an extremal linear subspace), but the converse deduction is also possible. In order to see this, it is sufficient to observe that the constant multiples of any  $f$  contained in  $(N^2)$  form a linear subspace of  $(N^2)$ .

4. The space  $(N^2)$  is complete.

In other words, if  $f_1, f_2, \dots$  is any sequence of functions contained in  $(N^2)$  and satisfying

$$\bar{M}(|f_j - f_k|^2) \rightarrow 0 \text{ as } j, k \rightarrow \infty, \quad (3)$$

then there exists in  $(N^2)$  a function  $f$  satisfying

$$\bar{M}(|f - f_j|^2) \rightarrow 0 \text{ as } j \rightarrow \infty. \quad (4)$$

The proof is a refinement, and at the same time a simplification, of the proofs of Besicovitch<sup>4</sup> and of Nalli.<sup>5</sup>

The limit relation (3) obviously implies the existence of an unbounded increasing sequence of positive numbers  $R(1), R(2), \dots$  such that

$$\lim_{n \rightarrow \infty} \sigma(n) = 0,$$

where

$$\sigma(n) = \text{fin sup}_{m > n, X > R(m)} \int_0^X |f_m(x) - f_n(x)|^2 dx / X, \quad (5)$$

if the fin sup refers to  $m$ , while  $n$  is fixed. Let  $n_1 < n_2 < \dots$  be a sequence of integers satisfying

$$\sum_{k=1}^{\infty} \sigma(n_k) < \infty. \quad (6)$$

Let  $g_k$  denote the  $n_k$ -th function of the sequence  $f_1, f_2, \dots$  and let  $S(k) = R(n_k)$ . Put  $f(x) = 0$  if  $0 < x < S(1)$  and  $f(x) = g_k(x)$  if  $S(k) \leq x < S(k+1)$ , where  $k = 1, 2, \dots$

It will first be shown that

$$\bar{M}(|f - g_k|^2) \rightarrow 0 \text{ as } k \rightarrow \infty. \quad (7)$$

To this end, let  $X$  be any number exceeding  $S(k)$ , where  $k$  is fixed. If  $i (\geq k)$  denotes the integer for which  $S(i) < X \leq S(i+1)$ , then

$$\begin{aligned} \int_{S(1)}^X |f(x) - g_k(x)|^2 dx &= \sum_{j=1}^{k-1} \int_{S(j)}^{S(j+1)} |g_j(x) - g_k(x)|^2 dx + \\ &\quad \sum_{j=k+1}^{i-1} \int_{S(j)}^{S(j+1)} |g_j(x) - g_k(x)|^2 dx + \int_{S(i)}^X |g_i(x) - g_k(x)|^2 dx. \end{aligned}$$

Hence it is seen from (6) and the definitions of  $g_j$  and  $S(j)$ , that

$$\int_{S(1)}^X |f(x) - g_k(x)|^2 dx / X \leq \sum_{j=1}^{k-1} \int_{S(j-1)}^{S(j)} |g_j(x) - g_k(x)|^2 dx / X + \sum_{j=k+1}^i \sigma(n_j).$$

Consequently, as  $X \rightarrow \infty$ ,

$$\bar{M}(|f - g_k|^2) \leq \sum_{j=k+1}^{\infty} \sigma(n_j),$$

and so (7) follows from (6).

It is clear that (4) follows from (7), and from the definition of  $g_k$ , in virtue of the triangular inequality.

It remains to be shown that  $f(x)$  is of class  $(N^2)$ . Clearly, the existence of (1) for  $g = |f_n|^2$ , where  $n = 1, 2, \dots$ , and (3) imply that

$$\mu = \lim_{n \rightarrow \infty} M(|f_n|^2) \quad (8)$$

exists as a finite limit. On the other hand, by Minkowski's inequality,

$$\left| \int_0^X |f(x)|^2 dx - \int_0^X |f_n(x)|^2 dx \right| / X < \int_0^X |f(x) - f_n(x)|^2 dx / X; \quad (9)$$

hence, if the integer  $n$  is fixed so that neither  $|\mu - M(|f_n|^2)|$  nor  $\bar{M}(|f - f_n|^2)$  exceeds a given  $\epsilon > 0$ , then, whenever  $X$  exceeds a bound depending on  $\epsilon$  and  $n$ ,

$$\left| \int_0^X |f(x)|^2 dx / X - \mu \right| < 2\epsilon.$$

Consequently, (1) exists for  $g = |f|^2$ , and

$$M(|f|^2) = \mu. \quad (10)$$

5. Let a function  $f(x)$ , where  $0 < x < \infty$ , be called of class  $(\bar{N}^2)$  if  $f(x)$  is of class  $(L^2)$  on every bounded interval  $(0, X)$  and  $\bar{M}(|f|^2)$  is finite. The functions of class  $(\bar{N}^2)$  will be considered to form a metric space carrying the same metric as that of the  $(N^2)$ -space; so that the  $(N^2)$ -space is a subspace of the  $(\bar{N}^2)$ -space.

*The  $(\bar{N}^2)$ -space is linear and complete.*

The linearity of  $(\bar{N}^2)$  follows from the inequality mentioned at the beginning (after the definition of  $\bar{M}$ ). On the other hand, the completeness is a consequence of the above proof, even though not of the final wording, of section 4.

6. It is clear that the above considerations can be adapted to any of the spaces  $(N^p)$ , where  $p \geq 1$ , which correspond to the space  $(N^2)$  in the same way as the classes  $(L^p)$  relate to the class  $(L^2)$ .

*If  $p \geq 1$  is arbitrary, the space  $(N^p)$  is complete but not linear.*

It is understood that, if  $p = 1$ , the class  $(N) = (N^1)$  is meant to be defined as consisting of those functions  $f$  which are of class  $(L) = (L^1)$  on every bounded  $x$ -interval and satisfy the condition that  $M(|f|)$  exists as a finite limit.

If  $p > 1$  and  $p^{-1} + q^{-1} = 1$ , then the product of two functions, one of which belongs to  $(B^p)$  and the other to  $(B^q)$ , belongs to  $(B) = (B^1)$ . But



this becomes false if  $(B^p)$ ,  $(B^q)$  and  $(B)$  are replaced by  $(N^p)$ ,  $(N^q)$  and  $(N)$ , respectively. This follows, even for  $p = 2 = q$ , from the above example proving that  $(N^2)$  is not linear. Correspondingly,  $(N^q)$  cannot be interpreted as the dual space of  $(N^p)$ , since such an interpretation would involve the definition of a scalar product.

7. Let  $(\bar{N}^p)$  denote the space which relates to the space  $(N^p)$  in the same way as the space  $(\bar{N}^2)$  relates to  $(N^2)$ .

If  $p \geq 1$  is arbitrary, the space  $(\bar{N}^p)$  is complete and linear.

The proofs are the same as before.

The situation can be summarized as follows:  $(B^p)$  is a subspace of  $(N^p)$  and  $(N^p)$  is a subspace of  $(\bar{N}^p)$ ; all three of these spaces are complete; the first and third of them are linear but the second is not.

<sup>1</sup> Actually, the definitions of Nalli (*loc. cit.*,<sup>2</sup> p. 306) are based on what results when the distance is assigned under the assumption that the upper limit occurring in (2) is replaced by the corresponding limit (which is required to exist). However, a glance at the proofs given by Nalli shows that this definition of her function space is equivalent to the above definition of the function space  $(N^2)_0$ .

<sup>2</sup> Nalli, P., *Rendiconti Circolo Matematico Palermo*, 38, 307, 318-319, 322-323 (1914).

<sup>3</sup> This fact has curious methodical consequences for a problem relating to the Riemann zeta-function; cf. Wintner, A., *Duke Mathematical Journal*, 10, 430 (1943), where the situation is explained in detail.

<sup>4</sup> Besicovitch, A. S., *Almost Periodic Functions*, Cambridge, 110-112 (1932).

<sup>5</sup> Nalli, P., *loc. cit.* <sup>2</sup> pp. 308-309.

## ON THE MAXIMUM PARTIAL SUM OF INDEPENDENT RANDOM VARIABLES

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Let  $X_n$ ,  $n = 1, 2, \dots$  be independent random variables with moments

$$E(X_n) = 0, \quad E(X_n^2) = 1, \quad E(|X_n|^3) = \gamma_n. \quad (1)$$

The reduction of the variance to 1 is not necessary, but is made here solely for the sake of simplicity. Let

$$S_n = \sum_{v=1}^n X_v, \quad S_n^* = \text{Max.}_{1 \leq v \leq n} |S_v|$$

It is a trivial observation that the same law of the iterated logarithm holds for  $S_n^*$  as for  $S_n$ . In particular one of Feller's theorems<sup>1</sup> becomes: If

$$\sup_n |X_n| = 0 \quad (n^{1/2}(\lg n)^{-1/2}), \quad (2)$$

then ("i. o." standing for "infinitely often").

$Pr(S_n^* > n^{1/2}[2lg_2n + 3lg_3n + 2lg_4n + \dots + 2lg_{p-1}n + (2 + \delta)lg_pn]^{1/2}$   
i. o.) is zero or one according as  $\delta$  is positive or not.

In the opposite direction Erdős has proved (oral communication) that there exist two constants  $c_2 > c_1 > 0$  such that

$$Pr(c_1 < \liminf n^{-1/2}(lg_2n)^{1/2}S_n^* < c_2) = 1.$$

His method, of an elementary nature, seems incapable of a sharper result. We shall show that if, besides (1), we assume that  $\gamma_n$  is e.g. bounded, then we can prove the following precise theorem which is the counterpart of Feller's theorem.

THEOREM. *We have*

$$Pr(S_n^* < \pi 8^{-1/2} n^{1/2} [lg_2n + 2lg_3n + lg_4n + \dots + lg_{p-1}n + (1 + \delta)lg_pn]^{-1/2}$$
  
i. o.)

equal to zero or one according as  $\delta$  is positive or not.

The assumption that  $\gamma_n$  is bounded can be considerably weakened; see the last paragraph of this abstract. However, the best possible condition has not been obtained.

We may also remark that the corresponding theorem for  $S_n$  is radically different and has been treated by Erdős and the author.<sup>2</sup>

We shall sketch the main lines of our method in a series of lemmas.

Let  $v_j = [jk^{-1}n]$ ,  $j = 1, \dots, k$ . Then  $(S_{v_1}, \dots, S_{v_k})$  is a random point in a  $k$ -dimensional space. Let its distribution function be  $F(u_1, \dots, u_k) = Pr(S_{v_1} \leq u_1, \dots, S_{v_k} \leq u_k)$ . Lemma 1 is an extension of Liapounoff-Berry theorem<sup>3</sup> to  $k$  dimensions and is proved by an extension of their methods. The condition regarding the third absolute moments is principally needed here. Notice that the dependence on  $k$  is essential.

LEMMA 1. *We have*

$$|F(n^{1/2}u_1, \dots, n^{1/2}u_k) - \Phi(u_1, \dots, u_k)| \leq Q_1 k^k n^{-1/2}$$

where  $Q_1$  (as  $Q_2, \dots$  later on) is a constant depending on the random variables but not on  $k, n$ , or the  $u_j$ 's; and  $\Phi(u_1, \dots, u_k)$  is the  $k$ -dimensional distribution function corresponding to the characteristic function

$$\exp. \left( -\frac{1}{2k} \sum_{j=1}^k (t_j + \dots + t_k)^2 \right)$$

We shall write  $\Phi(u) = \Phi(u, \dots, u)$ . Lemma 2 is an improvement on Erdős-Kac inequality.<sup>4</sup> The proof depends on Lemma 1 and also uses Liapounoff theorem.

LEMMA 2. *Let  $c > 0$ ,  $\psi_n > 0$  and  $n/\psi_n \uparrow \infty$ ,  $\epsilon_n \downarrow 0$ . Then we have*

$$\Phi\left(\frac{c}{\sqrt{\psi_n}}\right) + \frac{Q_2 k^k}{\sqrt{n}} > Pr\left(S_n^* < c \sqrt{\frac{n}{\psi_n}}\right) \geq \Phi\left(\frac{c - \epsilon_n}{\sqrt{\psi_n}}\right) - Q_2 \left(\frac{k^k}{\sqrt{n}} + e^{-\frac{\epsilon_n^2 k}{2\psi_n}} + \left(\frac{\psi_n}{\epsilon_n^2 n}\right)^{1/2}\right)$$

In order to evaluate  $\Phi(c\psi_n^{-1/2})$  we shall consider the special case where each  $X_v = \pm 1$  with probability  $1/2$ . In this case we find

LEMMA 3. If  $\alpha_n = o(\sqrt{n})$ , then

$$Pr(S_n^* < \alpha_n \sqrt{n}) = \frac{4}{\pi} \sum_{i=0}^{\infty} \frac{(-1)^i}{2i+1} \exp\left(-\frac{(2i+1)^2 \pi^2}{8\alpha_n^2}\right) + o\left(\frac{1}{\alpha_n \sqrt{n}}\right) = T(\alpha_n) + o\left(\frac{1}{\alpha_n \sqrt{n}}\right),$$

where  $T(\alpha_n)$  is defined by the series.

The proof of Lemma 3 starts with a combinatorial formula due to Bachelier,<sup>6</sup> and makes use of standard approximations together with a Fourier series transformation.

Combining Lemmas 2 and 3, and choosing, e.g.,

$$k = \frac{\lg n}{8 \lg_2 n}$$

we get

$$T\left(\frac{c + \epsilon_n}{\sqrt{\psi_n}}\right) + R_n \geq Pr\left(S_n^* < c \sqrt{\frac{n}{\psi_n}}\right) \geq T\left(\frac{c - \epsilon_n}{\sqrt{\psi_n}}\right) - R_n \quad (3)$$

where  $T(\alpha)$  is defined in Lemma 3 and

$$|R_n| \leq Q_3 \left( n^{-1/4} + \exp\left(-\frac{\epsilon_n^2 \lg n}{16 \psi_n \lg_2 n}\right) + \left(\frac{\psi_n}{\epsilon_n^2 n}\right)^{1/2} \right). \quad (4)$$

We may remark in passing that from (3) and (4) we can deduce a remainder term to the limiting distribution of  $S_n^*$ , e.g., if  $\alpha$  is a constant, then

$$Pr(S_n^* < \alpha \sqrt{n}) = T(\alpha) + o(\lg_2 n (\lg n)^{-1/2}).$$

Putting in (3) and (4)  $\epsilon_n \psi_n^{-1/2} = \eta_n$  and choosing, e.g.,  $\eta_n \geq 1600 \lg_2 n (\lg n)^{-1/2}$  we obtain

$$|R_n| \leq Q_4 (\lg n)^{-100}$$

If  $\epsilon_n \psi_n = o(1)$ , it is easily seen that there exist two constants  $A_2 > A_1 > 0$  such that

$$\frac{4}{\pi} e^{-\psi_n} (1 - A_1 \epsilon_n \psi_n) - \frac{4}{3\pi} e^{-\psi_n} \leq T \left( \frac{1}{\sqrt{\psi_n}} \left( \frac{\pi}{2\sqrt{2}} - \epsilon_n \right) \right) \leq$$

$$T \left( \frac{1}{\sqrt{\psi_n}} \left( \frac{\pi}{2\sqrt{2}} + \epsilon_n \right) \right) \leq \frac{4}{\pi} e^{-\psi_n} (1 + A_2 \epsilon_n \psi_n).$$

These estimates can be sharpened to a certain extent, but they are sufficient for our present purposes. We state the main result as follows.

LEMMA 4. If  $\psi_n \uparrow \infty$  and

$$\psi_n = o(\lg_2 n),$$

then,

$$Pr \left( S_n^* < \frac{\pi}{2\sqrt{2}} \sqrt{\frac{n}{\psi_n}} \right) = \frac{4}{\pi} e^{-\psi_n} (1 + o(1)).$$

Evidently the sequence,

$$\psi_n = \lg_2 n + 2\lg_3 n + \lg_4 n + \dots + \lg_{p-1} n + (1 + \delta)\lg_p n,$$

satisfies the conditions of Lemma 4.

Now we have the tools for proving our theorem. The proof for the case  $\delta > 0$  follows easily from Lemma 4 by taking a sequence  $n_k \sim \exp. (k/\lg k)$ . The proof for the case  $\delta \leq 0$  is much deeper and depends on the subtraction of a further subsequence with  $k_v \sim 4v \lg v \lg_2 v$ . We use in part arguments similar to Feller's, combined with estimates based on Lemma 4. It is impossible to indicate the method without going into details.

We state a more general theorem as follows. Let

$$s_n^2 = \sum_{v=1}^n \sigma_v^2, \quad \Gamma_n = \sum_{v=1}^n \gamma_v$$

and make the following assumptions:

$$\Gamma_n = o(s_n^2 \text{Max.}_{1 \leq v \leq n} \gamma_v^{1/2})$$

$$\text{Max.}_{1 \leq v \leq n} \gamma_v^{1/2} = o(s_n^{1-\theta}), \quad \theta > 0$$

Let  $\psi_n \uparrow \infty$ , then

$$Pr \left( S_n^* < \frac{\pi}{2\sqrt{2}} \frac{s_n}{\sqrt{\psi_n}} i. o. \right)$$

is equal to zero or one according as

$$\sum_{n=1}^{\infty} \frac{\sigma_n^2}{s_n^2} \psi_n e^{-\psi_n}$$

is convergent or divergent.

Detailed proofs of the above results will appear in another publication.

<sup>1</sup> Feller, W., "The General Form of the So-Called Law of the Iterated Logarithm," *Trans. Amer. Math. Soc.*, **54**, 373-402 (1943).

<sup>2</sup> Chung, K. L., and Erdős, P., "On the Lower Limit of Sums of Independent Random Variables," to appear in *Annals of Mathematics*.

<sup>3</sup> Berry, A. C., "The Accuracy of the Gaussian Approximation to the Sum of Independent Variates," *Trans. Amer. Math. Soc.*, **49**, 122-136 (1941).

<sup>4</sup> Erdős, P., and Kac, M., "On Certain Limit Theorems of the Theory of Probability," *Bull. Amer. Math. Soc.*, **52**, 292-302 (1946).

<sup>5</sup> Bachelier, *Calcul des probabilités*, t. 1, Paris (1912).

## SOME RESULTS ON ADDITIVE THEORY OF NUMBERS

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Let  $k$  be an integer  $\geq 11$ . Let  $r_{2s}(P)$  be the number of solutions of the system of Diophantine equations

$$x_1^h + \dots + x_s^h = y_1^h + \dots + y_s^h, \quad 1 \leq h \leq k \quad (1)$$

$$1 \leq x, y \leq P.$$

The author has proved that, for

$$s \geq k^2(2 \log k + \sqrt{\log k^2} + \frac{1}{2} \log \log k + 4), \quad (2)$$

the asymptotic formula

$$r_{2s}(P) \sim c_1 c_2 P^{2s - \frac{1}{2}(k+1)}, \quad (3)$$

holds (for  $P \rightarrow \infty$ ) where  $c_1$  is a constant defined by the integral

$$c_1 = \int_{-\infty}^{\infty} \dots \int_{-\infty}^{\infty} \left| \int_0^1 e^{2\pi i(\alpha_1 x^k + \dots + \alpha_s x^k)} dx \right|^{2s} d\alpha_1 \dots d\alpha_s$$

and  $c_2$  by a singular series.

If  $k < 11$ , the formula (3) is still true for  $s \geq s_0$ , where  $s_0$  is defined in the following table

$k$	2	3	4	5	6	7	8	9	10
$s_0$	4	9	24	63	157	381	890	2035	4596

This is a precise information about Tarry's problem.

The author has previously<sup>1</sup> established an asymptotic formula for the number of solutions of the system of Diophantine equations with prime unknowns

$$p_1^h + \dots + p_s^h = N_h, \quad 1 \leq h \leq k.$$

He has now succeeded in extending its range of validity from

$$s \geq 4.14k(k+1)(k+2) \log k \quad (s \geq 11)$$

to

$$s \geq k^2(4 \log k + 2 \sqrt{\log k^2 + \log \log k} + 9) \quad (s \geq 11). \quad (4)$$

Concerning Waring's problem Vinogradov<sup>2</sup> shows that the Hardy-Littlewood's asymptotic formula for the number of solutions of

$$x_1^k + \dots + x_s^k = N, \quad x_v \geq 0$$

holds for  $s \geq 20 k^2 \log k$ . His method is hardly able to go beyond the order of magnitude  $k^2 \log k$ , but the numerical factor may still be improved. Indeed the author has replaced Vinogradov's inequality by a sharper one

$$s \geq 4k^2(\log k + \frac{1}{2} \sqrt{\log k^2 + \frac{1}{4} \log \log k} + 1). \quad (5)$$

Vinogradov's asymptotic formula for Waring-Goldbach's problem also holds within the range (5).

<sup>1</sup> Hua, *Additive Prime Number Theory*. This booklet was accepted for publication by the Acad. of USSR in 1940. The appearance was delayed by World War II.

<sup>2</sup> *Comptes Rendus of USSR*, 1942, No. 7.

## ON THE MULTIPLICITY OF STEADY GAS FLOWS HAVING THE SAME STREAMLINE PATTERN

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The streamline pattern for any one steady flow of an ideal gas is the streamline pattern for a great many different modes of flow of such gas. Such trivial variants of the given flow as those obtainable by changing all pressures and densities in the same proportion are but special cases of wide classes of substitution flows having a common streamline pattern with the original flow. One such class of substitution flows of particular interest will be discussed here.

We shall consider steady flows of an ideal gas in which changes in entropy occur only in infinitely thin shock-front regions. This means that in regions between shocks the flow is *isentropic*, but not necessarily *homentropic*. That is, the entropy in a shock-free region is *constant along any given streamline* but is not necessarily *constant throughout the flow*. Only



if the flow is homentropic and not merely isentropic will the density be a unique function of the pressure throughout the shock-free region. The existence of such a function is the necessary and sufficient condition that the acceleration field,  $-\frac{1}{\rho} \text{grad } p$ , possess a potential (be irrotational).

Since irrotationality of the acceleration field is necessary (but not sufficient) for irrotationality of the flow field, only such isentropic flows as are also homentropic may possibly be irrotational. Hence in considering flows which are isentropic but not necessarily homentropic we include rotational as well as irrotational flows.

We shall first limit our attention to regions between shock fronts, and consider that class of substitution flows for a given steady streamline pattern for which the pressures remain unchanged. That is for which

$$p' = p \quad (1)$$

Now the dynamic equilibrium of the force components normal to the streamline requires that the normal component of the pressure gradient balance the centrifugal reaction of the flow, or

$$\frac{\partial p'}{\partial n} = \frac{\rho' v'^2}{R} \quad (2a)$$

$$\frac{\partial p}{\partial n} = \frac{\rho v^2}{R} \quad (2b)$$

where  $R$  represents the local radius of curvature of the streamline,  $v$  the flow velocity and  $\rho$  the mass density. Since  $p' = p$ , the condition

$$\rho' v'^2 = \rho v^2 \quad (3)$$

follows.

Now, for gases of constant specific heats,  $C_p$  and  $C_v$ ,

$$p \propto \rho^\gamma \exp. (s/C_v) \quad \left[ \begin{array}{l} \gamma \equiv C_p/C_v \\ s = \text{entropy (specific)} \end{array} \right] \quad (4)$$

For such gases the "velocity of sound" is given by

$$c \equiv \sqrt{\left(\frac{\partial p}{\partial \rho}\right)_s} = \sqrt{\frac{\gamma p}{\rho}} \quad (5)$$

and the Mach number, defined by

$$M \equiv \frac{v}{c} \quad (6)$$

becomes

$$M = \sqrt{\frac{\rho v^2}{\gamma p}} \quad (7)$$

Since  $p' = p$  and  $\rho'v'^2 = \rho v^2$ , the additional condition

$$M' = M \quad (8)$$

is obtained.

A further condition restricting the possible substitution flows is the necessity of dynamic equilibrium of the force components *along* each streamline. That is to say, Bernoulli's equation must hold along each individual streamline:

$$\frac{\gamma}{\gamma - 1} p' + \frac{1}{2} \rho' v'^2 = H' \rho' \quad (9)$$

where  $H'$  is a quantity (the "total enthalpy") which is constant along any particular streamline. The original flow satisfies a similar equation

$$\frac{\gamma}{\gamma - 1} p + \frac{1}{2} \rho v^2 = H \rho \quad (10)$$

Hence, from (1) and (3) it follows that

$$H' \rho' = H \rho, \text{ or } \rho' = \frac{H}{H'} \rho = m \rho \quad (11)$$

where  $m$  is a parameter constant along any given streamline but variable from streamline to streamline. Since there are no further conditions to be imposed on the substitution flows, we still have at our disposal the value of the arbitrary parameter,  $m$ , for each streamline.

*The streamline pattern and all pressures and Mach numbers are left unchanged if along each streamline the values of density and of velocity are multiplied respectively by  $m$  and  $1/\sqrt{m}$ , where  $m$  may change from streamline to streamline.*

This class of substitution flows has thus far in our discussion been limited to shock-free regions, but this restriction can now be removed. Consider the flow quantities immediately in front of and immediately behind a shock front to be designated by the subscripts 1 and 2. Then the necessary conditions of conservation of mass, momentum and energy for a streamline passing through a steady shock front can be expressed in the form of equations (12-14):

$$\rho_1 v_1 = \rho_2 v_2 \quad (12)$$

$$\rho_1 v_1^2 \sin^2 \alpha_1 + p_1 = \rho_2 v_2^2 \sin^2 \alpha_2 + p_2 \quad (13)$$

$$\rho_1 v_1^2 + K p_1 = \rho_2 v_2^2 + K p_2 \quad (14)$$

where  $\alpha$  represents the angle between the streamline and the shock front, and  $K$  is a constant depending on the kind of gas involved. It is clear that any substitution flow consistent with the requirements of equations (1), (3) and (11) will satisfy these shock conditions as well as does the original flow. Therefore this group of flow substitutions produces no change in streamline pattern even if shock fronts are present.

In the most general flows of an ideal gas the total enthalpy,  $H$ , ( $=i + \frac{v^2}{2}$ , where  $i$  is the specific enthalpy) is constant along each particular streamline, whether or not it intersects shock fronts, but it varies from streamline to streamline. The entropy is constant along each particular streamline in regions between shock fronts, but varies from streamline to streamline and increases discontinuously whenever the streamline crosses a shock front.

In supersonic regimes ( $v > c$ ) flows of this most general character can be computed by the use of the general method of characteristics combined with the shock-front integral relations (equations (12-14)) when suitable boundary conditions are imposed. However, the computation is considerably simpler if either the total enthalpy,  $H$ , or the entropy,  $s$ , is universally constant for a region under consideration. For if  $H$  is constant throughout the flow, a universal relation exists between  $v$  and  $c$ , as from (5) and (10):

$$\frac{c^2}{\gamma - 1} + \frac{v^2}{2} = H \quad (15)$$

On the other hand, if  $s$  is constant throughout a region of the flow, there exists a universal relation between  $p$  and  $\rho$ . (See equation (4).)

By the use of the class of substitution flows discussed above, a flow of non-constant  $H$  and  $s$  can be readily replaced by a substitution flow having either constant  $H$  or (in a shock-free region) constant  $s$  if the values of  $m$  are suitably chosen on any surface intersecting each streamline a single time. The simpler substitute flow would then yield directly the pressures, Mach number and flow pattern of the original flow and, after a simple inversion of the conversion from original to substitute flow, the densities and velocities as well.

Similarly, if it is desirable to do so, an original flow problem involving non-constant  $H$  and constant  $s$  could be replaced by one having constant  $H$  and non-constant  $s$ , and vice versa. It is not possible, however, to substitute a flow having both  $H$  and  $s$  constant for one in which either is non-constant. In other words, this class of substitution flows does not in general allow the replacing of a rotational flow by an irrotational one.

The relations discussed may be arrived at more briefly, but less directly, from dimensional considerations. Along each stream tube, there exists for a perfect gas only one independent dimensional reference quantity, as for instance the "reservoir" pressure. All variables can be expressed in terms of that reference pressure in conjunction with one non-dimensional quantity as for instance the local Mach number. Thus the local pressure is equal to the reference pressure multiplied by a function of the Mach number, and of non-dimensional quantities representing the geometry involved. Hence the lateral pressure gradient is determined and invariant with respect to transformations not involving changes of the geometry or changes of the reference pressure and thus changes of any pressure. It follows that it must be possible to write all pertinent equations in terms of the local pressure, the local Mach number and the space coördinates, thus eliminating one dependent variable.

Among possible flows calling for application of these relations, there are jets from different "reservoirs" flowing together. Such is the flow when a propulsion jet issues into the rapidly moving air (relative to a missile or airplane) of the atmosphere. It may also be instructive to idealize boundary layer wakes by considering them as jets of a perfect gas with a lowered total enthalpy.

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## TURBULENCE AS AN ENVIRONMENTAL DETERMINANT OF RELATIVE GROWTH IN DAPHNIA

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Temporal variation or cyclomorphosis is pronounced in many limnetic races of *Daphnia*. The relative length of the head is the most variable aspect of such races of the north temperate zone. The winter and early spring generations bear short round heads resembling those characteristic of pond *Daphnia*. Individuals of midsummer generations have elongate heads, called helmets, which are often nearly as long as the rest of the body. During the period of these striking phenotypic changes reproduction is entirely asexual, which means that the genetic constitutions of all generations are almost identical. These phenotypic differences must therefore be determined by seasonally variable cytoplasmic or environmental factors, in all probability the latter.

The aim of the present investigation is precise determination of all of the environmental factors controlling cyclomorphosis. The efforts of

students in the first two decades of this century were directed toward finding a single environmental variable which controlled the relative head size. Wesenberg-Lund<sup>1</sup> concluded that temperature was the controlling factor while Woltereck<sup>2</sup>, denying this, claimed that the level of nutrition was the prime control. The experiments of Coker and Addlestone<sup>3</sup> in 1938 indicated, however, that different environmental factors were effective at different periods of the life history of an individual. They showed that temperature did control head size, but only during the embryonic period, some unisolated factor being involved during postnatal life. The author<sup>4</sup> was able to confirm these findings of Coker and Addlestone on several species.

The rate of relative helmet growth during postnatal life can be measured by the value of  $k$  in Huxley's<sup>5</sup> allometric equation,  $y = bx^k$ , as was demonstrated in the study<sup>4</sup> of the population of *D. retrocurva* Forbes in Bantam Lake, Connecticut (1945). From April until mid-June head ( $y$ ) and carapace ( $x$ ) grew at the same relative rate ( $k = 1$ ). Thereafter, helmet growth was tachyauxetic, the rate reaching a maximum ( $k = 1.44$ ) a month later. It was still greater than unity ( $k = 1.27$ ) at the end of the series in mid-August. Tachyauxetic growth occurred that season only when the water was above 18–19°C., but above this threshold appeared to be independent of both temperature and nutrition. Individuals of this population were cultured in laboratory vessels under conditions of temperature and nutrition which supported tachyauxetic helmet growth in the lake. Yet the helmets always grew relatively more slowly ( $k = 0.52$  to  $0.74$ ) than the rest of the body in these laboratory cultures. An investigation of the effects of size of vessel<sup>6</sup> and population density<sup>4</sup> proved that they are not responsible for this difference between the rates of relative helmet growth in the lake and in laboratory cultures. An experimental study of the rôle of turbulence was more fruitful and forms the subject of this report.

The population of *D. retrocurva* in Bantam Lake was so small in the summer of 1946 that it was impossible to collect any experimental animals of this species. The substitution of another species was necessary, so a race of *Daphnia longispina* with apicate helmets, also present in Bantam Lake, was utilized. The shape of the helmets of this race in mid-summer can be judged from the right-hand column of figure 2. Individuals from this population were used in the investigation of the influence of population density and size of vessel, as well as that of turbulence on the rate of postnatal relative helmet growth. Some of the *Daphnia* collected on September 2 were preserved and the remainder were brought back alive to the laboratory. Twenty adults with eggs or embryos in the broodpouch were selected as parents. These adults were divided into two equal groups. One was to be maintained in turbulent water while the control group was kept in



the usual quiet water of laboratory culture vessels. The cultures were kept in two similar rectangular museum jars of dimensions  $20 \times 23 \times 30$  cm. Each held ten liters of Bantam Lake water, which had been twice filtered through  $10 \times$  bolting silk. The jars were placed side-by-side under fluorescent lights in a room with a fairly constant temperature. A small electric stirrer rotated a nearly straight glass rod which extended about ten centimeters below the surface of the water. The turbulence so produced was judged to be sufficient when the *Daphnia* were swept around by the current. Measurement of the amount of turbulence was not possible. The nanoplankton of the lake water reproduced rapidly enough to serve unaugmented as a sufficient source of food.

Periodic microscopic examination of the adults showed that all of them lived and reproduced in each culture. No measurements of their offspring were made during the first two weeks. The temperature fluctuated slowly between  $17.2^{\circ}\text{C}$ . and  $23.1^{\circ}\text{C}$ . The mean of the daily readings for the turbulent culture was  $19.4^{\circ}\text{C}$ ., for the control  $19.3^{\circ}\text{C}$ . At the end of this period those offspring of each culture which had matured were measured and drawn.

The difference in relative head length in the two cultures was remarkable. While the heads in the controls were of the same relative length as in all previous laboratory cultures, those reared in turbulent water had relatively longer helmets, more nearly like those found in the lake. A representative adult from this first generation of each culture is drawn in figure 2.

Accumulation of sufficient data for a determination of the relative rates of helmet growth in the two cultures was highly desirable. All the instars of the first generation constituting the population at the end of the period could not be grouped to provide this information as the temperature, hence the relative head length at birth, had been too variable. It was therefore necessary to attempt a more rigorous temperature control. The two vessels were placed side-by-side in a 60-liter water bath. First generation adults were used in this experiment. Eight from the turbulent culture were replaced in turbulent water. Ten from the control culture were replaced in non-turbulent water. The temperature in each remained between  $19.4^{\circ}\text{C}$ . and  $22.4^{\circ}\text{C}$ . during this sixteen-day experimental period, except on the first day when it reached  $23.0^{\circ}\text{C}$ . Temperature fluctuations were simultaneous in the two cultures. The mean of the sixteen daily temperature records in each culture was  $21.2^{\circ}\text{C}$ . Nutrition as judged by the color of the guts was very good during the early phase of the experiment and slowly decreased so that at its termination, although adequate, it was poorer than at the outset. This change followed the same course in both vessels. It should be emphasized here that the relative rate of helmet growth is not influenced by changes in the nutritive level.



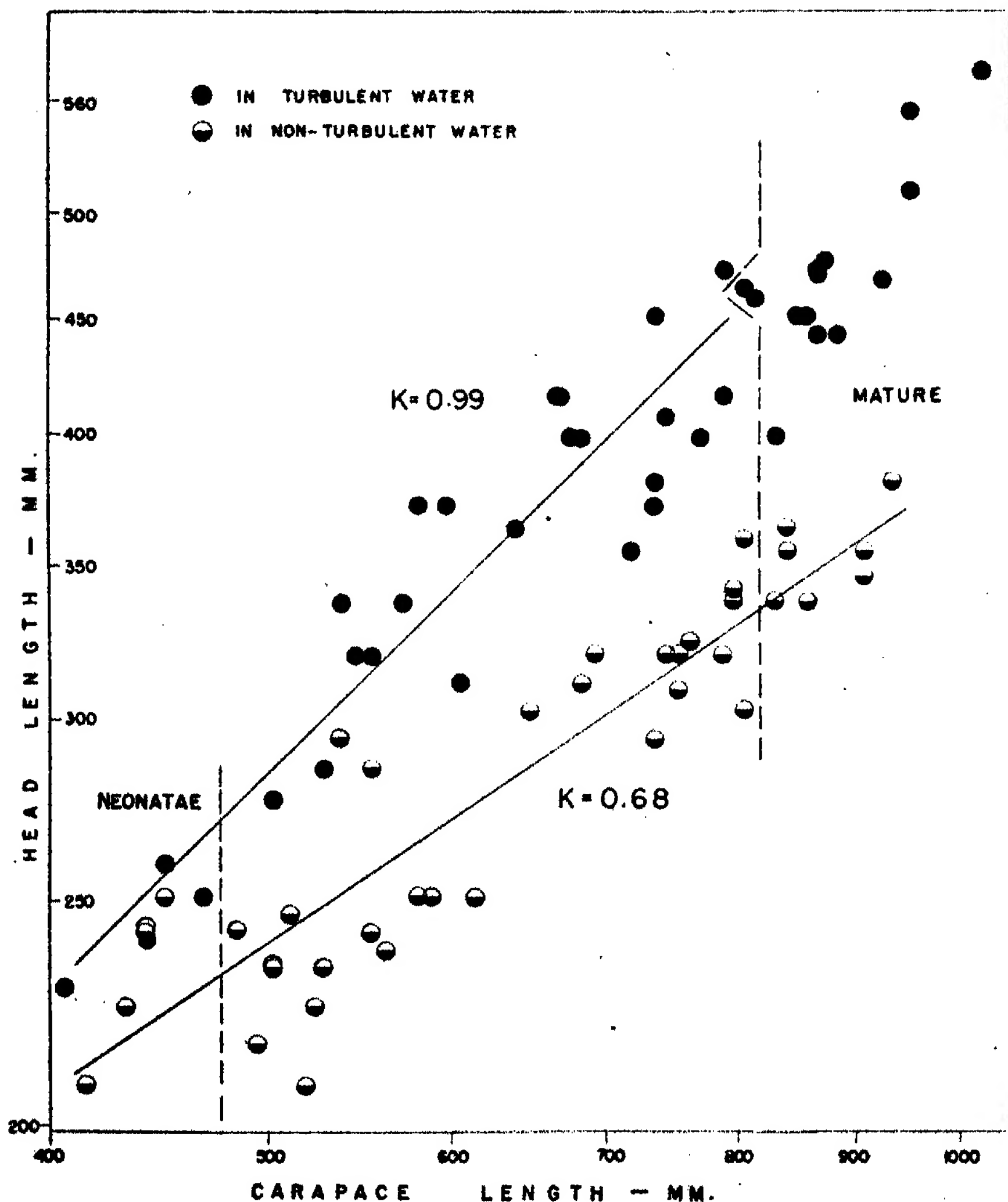


FIGURE 1.

Comparison of rates of relative head growth (*Daphnia longispina*, Bantam Lake, Conn.) in laboratory cultures with turbulent and non-turbulent water. See text for details.

Periodically a portion of the *Daphnia* population was removed from the culture vessels for examination. Each specimen was anesthetized with ethyl urethane, measured, and drawn with the camera lucida. These animals if properly treated, appeared to suffer no ill effects and were returned to the cultures. The measurements of head length and carapace length were made with an ocular micrometer. The head length was then plotted on a logarithmic grid against carapace length for each individual.

This gives a linear array of points for each culture. Measurement of the slope of the line of best fit for each array provides the  $k$  value for each culture. The details of measuring and analysis of measurements are given elsewhere.<sup>4</sup> When the pre-adult rate of relative helmet growth is isauxetic or tachyauxetic, there is an appreciable fall in the rate after maturity. No change in rate is apparent when early growth is bradyauxetic. The exponent,  $k$ , for immature specimens in the turbulent water is 0.99. Calculated for all specimens in non-turbulent water it is 0.68. (Fig. 1.)

This difference between the rates of relative helmet growth in turbulent and non-turbulent cultures could be either a direct effect of turbulent water movements on the *Daphnia* or an indirect one. Turbulence might act indirectly by increasing nutrition or oxygen concentration. Yet, as pointed out above, the difference between the levels of nutrition in the two cultures at any time was small when judged by microscopic examination of the guts of the transparent living animals. Oxygen determinations using the Winkler process showed that the water in both vessels was saturated with oxygen at the end of the experiment. This leaves a direct effect of turbulence as the more likely explanation, but no definite information is available as to the manner in which it influences the growth processes of *Daphnia*. It is possible that there are environmentally conditioned differences in metabolic rate associated with different rates of relative helmet growth. This would parallel the situation in *Locusta migratoria* where the externally determined solitary and migratory phases are known to have significantly different rates of oxygen consumption.<sup>7</sup>

Adult *Daphnia* reared in turbulent water in the laboratory have helmets relatively larger than those reared in non-turbulent cultures but they do not attain the proportions of adults which developed in the lake at about the same temperature. A comparison of development in these three environments is given in figure 2. These camera lucida drawings are all to the same scale, except for the neonatae which have twice the linear magnification. The left and center columns contain specimens taken from the non-turbulent and turbulent laboratory cultures, respectively, while that on the right shows specimens in the Bantam Lake population at the time when the parents for the experimental cultures were collected. These parents had the appearance of the adult at the top of that column. The adults of similar size which head the other two columns are the offspring of these females which were reared under turbulent and non-turbulent laboratory culture. Development in the two laboratory cultures will first be considered. The relation of each specimen to the rest of the population samples can be checked in figure 1. More than one specimen is drawn for most size groups to illustrate the range of variation. Specimens toward the left of either column have relatively smaller helmets, appearing below the line of best fit in figure 1. Those to the right have relatively larger helmets

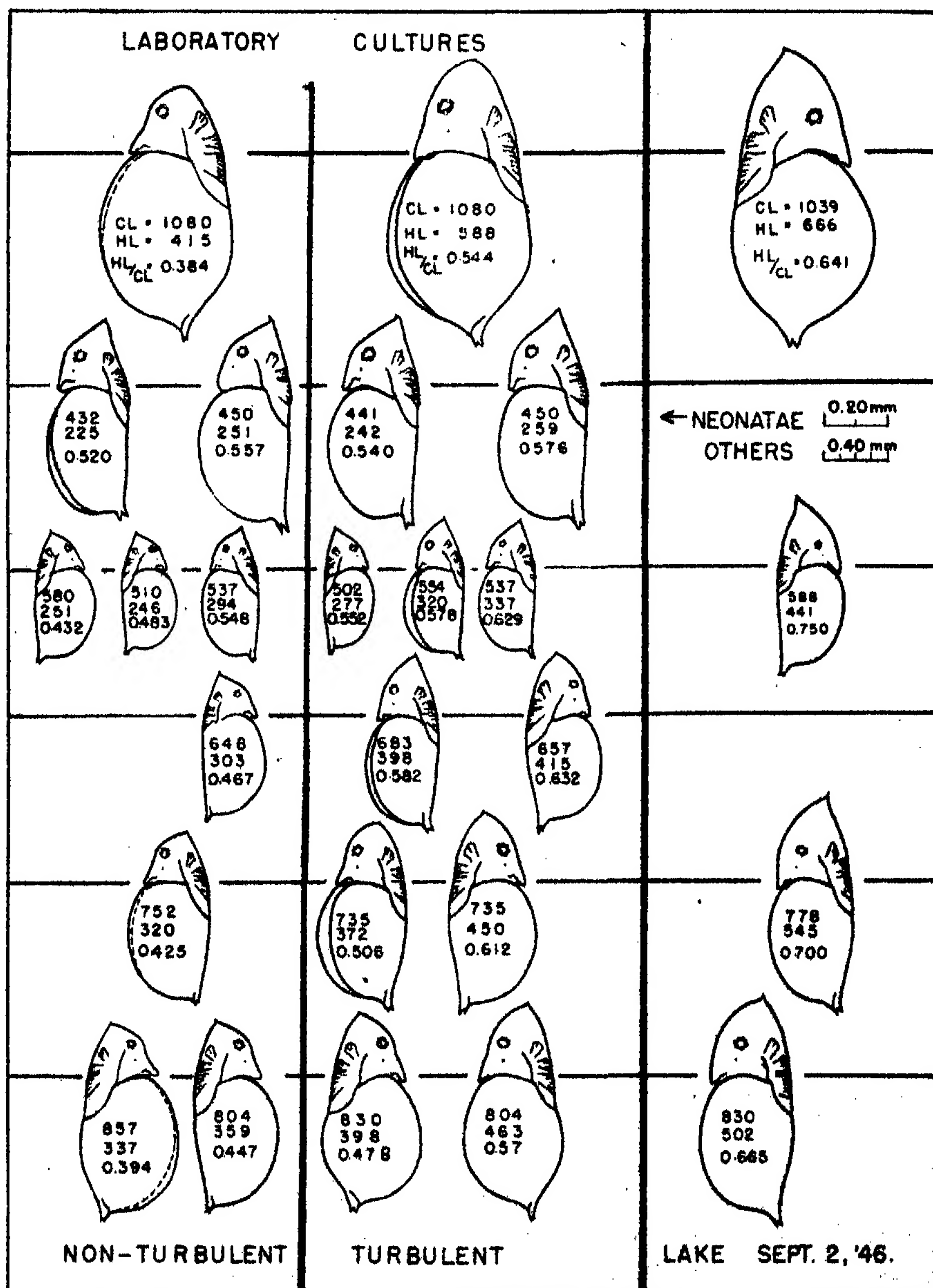


FIGURE 2.

Camera lucida drawings illustrating differences in helmet development in individuals from the same population of *Daphnia longispina* (Bantam Lake, Conn.) when reared in three different environments. The range of temperature and nutritive conditions was the same in all three. See text for details.

and those in the middle correspond to points on the curve. The relative helmet size of each *Daphnia* is given as a ratio. The effect of the different relative growth rates is apparent even on comparison of the neonatae with the next larger size group. While the newborn of the two cultures are identical, the lowest HL/CL ratio for specimens of the next larger size group in the turbulent culture is as high as the highest among the same sized individuals of the control culture. Among larger specimens the lowest HL/CL ratios in the turbulent culture are greater than the highest found in the same size group from non-turbulent water. The younger individuals in the right-hand column represent the only specimens of the appropriate instars to be found in the sparse population in Bantam Lake at that time. Unfortunately no neonatae were taken. As the temperatures at the surface, at one meter's depth and just above the bottom were 22.7°C., 21.6°C. and 20.2°C., respectively, the neonatae undoubtedly had helmets of about the same relative size as those in the laboratory cultures. If so, the helmets must have been growing relatively more rapidly than those in the turbulent laboratory culture in order to produce the higher helmets in the later instars. Although the absence of representatives of the early instars precludes an accurate determination of the relative growth rate, comparison with the laboratory growth rates should provide an approximation. The helmets in the turbulent laboratory culture were growing at the same relative rate as the rest of the body (isauuxesis) since the slope of the line best fit is essentially unity ( $k = 0.99$ ). Relative helmet growth in the lake must therefore, have been tachyauuxetic ( $k > 1$ ).

The information which this experiment provides concerning the relative rates of helmet growth under different environmental conditions is incomplete, yet it invites integration with what we know of the seasonal differences in relative growth rates in *D. retrocurva*. The helmets of the Bantam Lake population of the latter species grew isauxetically from the first of April to the middle of June 1945, under a wide range of nutritive and thermal conditions. The race of *Daphnia longispina* in the turbulent laboratory culture, well fed and at somewhat higher temperatures exhibited isauxetic helmet growth. This suggests that turbulence of the lake waters may be the factor permitting isauxesis. If so, it seems probable that turbulence is also necessary for tachyauuxetic growth. Differences in the amount of turbulence might determine the rate of relative helmet growth or turbulence may be a necessary, but not a sufficient environmental factor for tachyauuxesis. Further laboratory experimentation will explore this possibility. There is also hope of estimating the seasonal variation in the amount of turbulence in lakes.

One remark about the significance of turbulence in the interpretation of the biology of limnetic *Daphnia* should be made. Should turbulence prove to be a necessary environmental condition for the development of helmets

in many races it would explain the oft-noted restriction of helmeted populations to the upper, turbulent waters of stratified lakes. Those living in lower, less turbulent waters usually have shorter helmets.

Summary.—Preliminary experiments on a cyclomorphic race of *Daphnia longispina* indicate that turbulence of the water is one of the environmental factors controlling relative rate of helmet growth during postnatal life.

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## A DIRECT DEMONSTRATION OF THE PHOSPHORUS CYCLE IN A SMALL LAKE\*

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It is well known that the total quantity of plankton present in the waters of a lake may undergo marked and rapid variation, so that in the course of a year a number of pulses or maximum populations may succeed each other. Juday and Birge<sup>1</sup> noted that such rises in the phytoplankton might occur without reducing the phosphate content of the water and that on occasions both phosphate and plankton might rise together. It has also been noted for example by Pearsall,<sup>2</sup> that rises in the population of blue-green algae may occur at the end of summer when it would seem that the phosphorus content of the water was totally inadequate to support an increased phytoplanktonic population. In an earlier paper<sup>3</sup> much indirect evidence was assembled indicating that in Linsley Pond, a small inland lake which develops a very stable thermal stratification in summer, there is a continual liberation of phosphorus from the mud into the free water. Such of this phosphorus as enters the illuminated layers of the lake is believed to be taken up by the phytoplankton, later to be sedimented as a fine rain of particulate matter, partly no doubt dead phytoplankton, but also feces of



zooplankton feeding on the plant cells. The resultant movement of phosphorus is thus believed to be a horizontal movement into the free water as phosphate, and a vertical downward movement as seston or particulate matter. The very low concentrations usually observed in summer in the surface waters of lakes are thus to be regarded as steady state concentrations, maintained at low levels by the activity of the phytoplankton, the rate of development of which depends rather on the rate of supply of phosphate ions from the mud than on their concentration in the free water. Such an hypothesis explains the rather paradoxical situations encountered by other workers and is in accord with the facts relating to chemical cycles in Linsley Pond and other lakes. If the hypothesis is correct, phosphorus present in the surface as phosphate on any given day should move to greater depths during the course of the succeeding few days or weeks, even in fully stratified lakes in which virtually no mixing is taking place. The possibility of obtaining relatively large amounts of the radioactive isotope of phosphorus  $P^{32}$  permits the hypothesis to be tested.

On June 21, 1946, a sample of radiophosphorus, of strength approximately ten millicuries, received as phosphoric acid and made up as sodium phosphate in hundredth normal sodium bicarbonate, was introduced into the surface water of Linsley Pond in twenty-four approximately equal portions. Twelve of the portions were placed at approximately equal distances along a line running across the middle of the lake from West to East. The other twelve samples were placed along a line between the first line and the outlet at the south end of the lake. As a light south wind was blowing at the time, and as the surface water of the lake could be seen to be drifting northward when the boat was anchored in the middle of the lake, it was believed that this distribution of the samples would secure a fairly uniform dispersion of radiophosphorus in the circulating surface waters.

Collections of water were made on June 28 in the deep central part of the lake normally used for limnological stations. For measurement of radioactivity, the vertical water column was divided into four layers, from each of which approximately 18 liters of water was collected, as is indicated in table 1. The depths of collection refer to the position of the top of the 1.25-liter Nansen reversing bottle used to collect the water, the figures after the depths indicate the number of times the bottle was filled at each depth. The deepest water to be included at each filling of the bottle came from approximately 50 cm. below the top of the bottle so that the composite samples may be regarded as representing I, 0-3 m.; II, 3-6 m.; III, 6-9 m. and IV, 9-14.5 m. layers. The composite sample from each layer was evaporated almost to dryness, the organic matter oxidized with nitric and perchloric acids, and after addition of a drop of phosphoric acid as a carrier, the phosphate was precipitated as ammonium phosphomolyb-



date. The measurements of radioactivity were performed on the dry phosphomolybdate precipitates on filter paper.

Owing to the great dilution of the radioactive material, it was necessary to make a large number of counts, particularly on the sample from layer III, and to test the significance of the means obtained by Fisher's<sup>4</sup> table of *t*. The voltage stabilizer of the only Geiger counter circuit available was not sufficiently good to prevent alterations in the background count which completely obscured the increases, in single two-minute counts, due to the radioactivity of the samples. In the case of the samples from layers I and II, the mean count was found to be significantly different from the background when twenty-one 2-minute counts had been made; for quantitative determination nine more counts were included. For the lower activity of the sample from layer III, sixty-three runs of two minutes, alternated with background counts on a control sample of phosphomolybdate

TABLE 1

	LAYER	COMPOSITION OF SAMPLE	COUNTS PER M. <sup>2</sup> PER MIN.	PROBABILITY DUE TO CHANCE	VOL. LAYER M. <sup>3</sup>	COUNTS PER LAYER PER MIN.
I	0-3 m.	0.0 m., 2; 0.5 m., 3 1.0 m., 3; 1.5 m., 2 2.0 m., 3; 2.5 m., 2	1100	0.01-0.001	241,800	266.10 <sup>4</sup>
II	3-6 m.	3.0 m., 2; 3.5 m., 2 4.0 m., 3; 4.5 m., 2 5.0 m., 3; 5.5 m., 2	980	0.01-0.001	192,400	189.10 <sup>4</sup>
III	6-9 m.	6.0 m., 2; 6.5 m., 2 7.0 m., 3; 7.5 m., 2 8.0 m., 3; 8.5 m., 2	620	0.02-0.01	120,400	75.10 <sup>4</sup>
IV	9-14.8 m.	9.0 m., 4; 10.0 m., 3 11.0 m., 2; 12.0 m., 2 13.0 m., 2; 14.0 m., 1	(200	0.5-0.6)	Sum of sig- nificant values	530.10 <sup>4</sup>

precipitated in concentrated oxidized surface water collected on June 13, 1946, were made. The counts for layer III only appear significant if the significance of the mean difference between each run and the immediately preceding control background run be considered. This is reasonable, since the voltage will vary little between two consecutive counting periods, but over the whole sixty-three pairs of observations the over-all changes in voltage will greatly increase the variance of both background and sample counts. The sample from layer IV gave no significant indication of radioactive phosphorus after forty-two observations had been made and must be regarded as negative. With improved counting facilities and a somewhat larger quantity of radiophosphorus it may be possible to measure the activity of phosphate phosphorus, organic soluble phosphorus and sestonic phosphorus separately and to follow the cycle for longer periods on some future occasion.

The results of the study are set out in table 1, all counts being adjusted for decay and fluctuation of the counting rate, to correspond to the time and conditions of measurement of a phosphomolybdate preparation of an aliquot of  $10^{-6}$  of the original sample, which gave a count above the background of 715 per minute on July 8. On this basis the total quantity of  $P^{32}$  put into the lake corresponds to  $715 \cdot 10^6$  counts per minute. The total recovery therefore amounts to 74.2% of the  $P^{32}$  introduced.

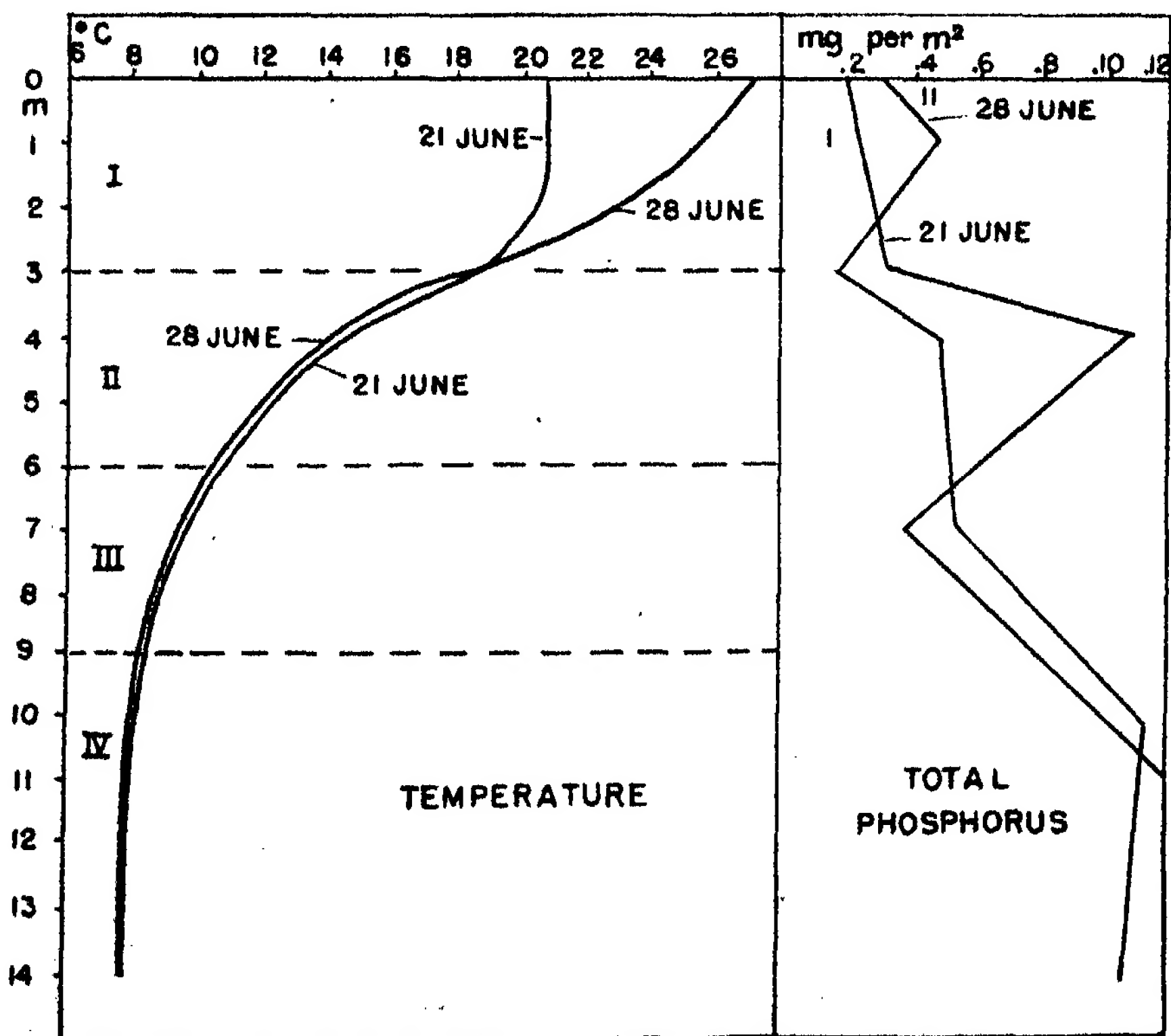


FIGURE 1.

Vertical distribution of temperature and total phosphorus in Linsley Pond 21 and 28 June, 1946, and limits of the four layers used in the present study.

The temperature curves (fig. 1) for the days at the beginning and end of the experiment are almost identical below three meters. The slightly lower temperature in the deep water on June 28 is certainly insignificant, and due to slight distortion of the isotherms by wind, as frequently occurs in all lakes. The chief change in the week of the experiment was a great heating of the surface water due to a sudden spell of hot weather.

Determinations of total phosphorus indicate a movement of phosphorus into the epilimnetic layer I during the period under consideration. The apparent decline in the total phosphorus at 4 m. in layer II is probably due to the descent of decomposing plankton which was clearly visible in the 4 m. sample on June 21, but which was probably at a greater depth and was consequently missed in sampling on June 28.

In spite of the fact that phosphorus has entered the epilimnion during a period when there can have been practically no vertical mixing, it is clear from the radioactivity measurements that 47% of the phosphorus present as phosphate in solution at the surface on the first day of the experiment had descended into the stratified part of the lake below 3 m. and about 10% had crossed the 6 m. level. These findings completely confirm the hypothesis put forward in 1941 and summarized in the first paragraph of the present contribution.

Of the 25.8% of the  $P^{32}$  put into the lake and not recovered, the greater part had probably entered the aquatic plants and sediments in contact with the 0-3 m. layer. Assuming uniform sedimentation, the bottom of the lake from the 0 m. to 3 m. contour, having an area of about 24.6% of the surface area of the lake, would have received a like fraction of the radiophosphorus. Actually uniform vertical sedimentation is unlikely in the shallow water, but an appreciable amount of radiophosphorus entered the aquatic vegetation growing in the littoral zone. Littoral plants, mainly *Potamogeton* spp., were collected on June 28 and July 12 and showed the activities in counts per minute, given in table 2.

TABLE 2

	PER GRAM WET	PER GRAM DRY (80°C.)	PER GRAM P.
June 28	1.65	8.85	4700
July 12	0.68	4.47	2800

If the wet weight be taken as approximately equal to the volume it will be observed that on June 28, when the surface water gave a count of the order of  $10^8$  per m.<sup>3</sup> per minute, the weed gave a count of the order of  $10^6$  per m.<sup>3</sup> per minute, indicating a thousand-fold concentration of radiophosphorus in the weed over that in the water. It is unfortunate that no quantitative data are available on the population of littoral plants in Linsley Pond, though, except at the south end, no extensive beds occur. The results just given suggest that in lakes in which there are wide expanses of littoral vegetation, this vegetation competes with the phytoplankton for phosphorus. Such competition is of course likely to be most severe during the growth period of the plants from April to June, and may conceivably account for phytoplankton minima often observed at the end of May or June.

\* The authors wish to acknowledge their indebtedness to Dr. E. F. Pollard for his help and advice and for arranging for the manufacture of the sample of P<sup>32</sup> employed. An unsuccessful attempt to perform an experiment of the kind described was made in 1941 by Pollard, Hutchinson and W. T. Edmondson.

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# PROCEEDINGS OF THE NATIONAL ACADEMY OF SCIENCES

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## *KYNURENINE AS AN INTERMEDIATE IN THE FORMATION OF NICOTINIC ACID FROM TRYPTOPHANE BY NEUROSPORA\**

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For a number of years it has been clear that nicotinic acid is synthesized by at least some mammals. The amount of this vitamin retained in the body of a rat plus that excreted may far exceed the dietary intake.<sup>1-3</sup> On a diet containing adequate protein the dog appears not to need exogenous nicotinic acid.<sup>4</sup> Recently it has been shown that nicotinic acid synthesis in the rat,<sup>5, 6-7</sup> man,<sup>8, 9</sup> the horse<sup>10</sup> and the cotton rat<sup>10</sup> is increased when the diet is high in tryptophane.

It has likewise been shown by Woolley<sup>11</sup> and by Kodicek, Carpenter and Harris<sup>12</sup> that nicotinic acid and tryptophane are interchangeable in counteracting the pellagragenic effects of 3-acetyl pyridine, indole-3-acetic acid, and a factor present in corn that may be related to one of these.

As pointed out by Rosen, Huff and Perlzweig<sup>5</sup> these facts suggest that tryptophane may serve as natural precursor of nicotinic acid. Other interpretations are possible, however.<sup>13</sup>

Evidence in support of the hypothesis that tryptophane is a precursor of nicotinic acid and that kynurenine is an intermediate in the conversion comes from a study of a mutant strain of *Neurospora crassa*. This mutant strain, designated 65001, descended from a conidium irradiated with 9400 ergs of ultraviolet per square millimeter. The manner of treatment and of isolating and detecting mutant strains has been described elsewhere.<sup>14</sup>

Mutant strains of *Neurospora* requiring exogenous tryptophane for growth are known.<sup>15</sup> Other strains have been studied that will not grow unless nicotinic acid or a related compound is supplied in the medium.<sup>16</sup> In certain of these strains the requirements are specific. Tryptophaneless strain 10575 will not respond to nicotinic acid, and nicotinic acid-requiring strains 3416 and 4540 will not grow on tryptophane. Strain 65001, on the other hand, grows if supplied with *either* tryptophane or nicotinic acid.



In outcrosses to wild-type strains, mutant 65001 behaves as though it differed from normal by a single gene. Asci from the cross carry four ascospores that give rise to cultures that grow in the absence of both tryptophane and nicotinic acid and four spores that give cultures which, like the mutant parent, require either but not both of these growth factors. The gene in which 65001 and wild type differ is not sex linked. Its genetic relation to genes carried in mutant form by previously reported tryptophaneless and nicotinicless strains has not yet been completely established.

The behavior of strain 65001 suggested to us that it, like the rat, might be able to make nicotinic acid from tryptophane. Kynurenine, known as a product of tryptophane metabolism in mammals,<sup>17, 18</sup> insects<sup>19, 20</sup> and bacteria,<sup>20</sup> suggested itself as a possible intermediate in this process. Tests of *l*-kynurenine, isolated from the urine of rabbits and kindly made available to us by Professor S. Lepkovsky of the University of California, showed it to be active in promoting growth of mutant 65001. As determined by three-day growth in 125-ml. flask cultures,<sup>16</sup> approximately 1.6 micromoles of *l*-kynurenine gives half-maximum growth. The corresponding values for nicotinamide and *l*-tryptophane are 1.4 and 9.8 micromoles, respectively.

Following the method of Butenandt, *et al.*,<sup>21, 22</sup> *dl*-kynurenine was synthesized. It proved to have half the activity of the natural material showing that the *d*-isomer is not utilized as a growth factor by 65001.

Like the mammal, strain 65001 produces an excess of nicotinic acid (or amide) when grown in the presence of an excess of tryptophane but not when grown on an amount just sufficient for growth. Thus four 500-ml. flask cultures of 65001, made up with 100 ml. of culture medium each, two containing 2 mg. *dl*-tryptophane per 100 ml. medium and two containing four times as much, were grown for seven days and the culture medium tested for nicotinic acid. The lesser amount of tryptophane was adequate for growth of strain 65001. Following replenishment of sugar and biotin, the culture media were tested for ability to support growth of mutant 3416, a strain which appears to be specific for nicotinic acid or nicotinamide.<sup>16</sup> The media initially high in tryptophane gave good growth while those initially low in tryptophane gave very little. While it is conceivable that the compound accumulated is not nicotinic acid or nicotinamide but a compound of unknown nature with similar biological activity, this seems unlikely.

An experiment made in a similar manner shows that when 65001 is supplied *dl*-kynurenine in an amount just sufficient for maximum growth (500 micrograms per 20-ml. medium), no nicotinic acid is given off in the culture medium in 12 days. But when the kynurenine is increased fourfold, nicotinic acid accumulates in the culture medium in an amount sufficient for maximum growth of strain 3416—at least 0.5 microgram per milliliter of culture medium.

These experiments lend strong support to the view that *Neurospora* converts tryptophane to nicotinic acid by way of kynurenine.

Since kynurenine is active as a growth factor for strain 39401 which was used as an assay strain by Bonner and Beadle<sup>16</sup> in the isolation of two nicotinic acid precursors accumulated by strain 4540, the question arises as to the relation of kynurenine to these precursors. Elementary analyses of the strain 4540 precursors reported by Bonner and Beadle show that they cannot be kynurenine, a conclusion consistent with independent evidence indicating that they both contain a pyridine ring.<sup>16</sup> A reexamination of the properties of mutant 39401 shows that it, like mutant 65001, grows when supplied tryptophane. These two strains may be alike genetically; attempts to cross them have so far been unsuccessful.

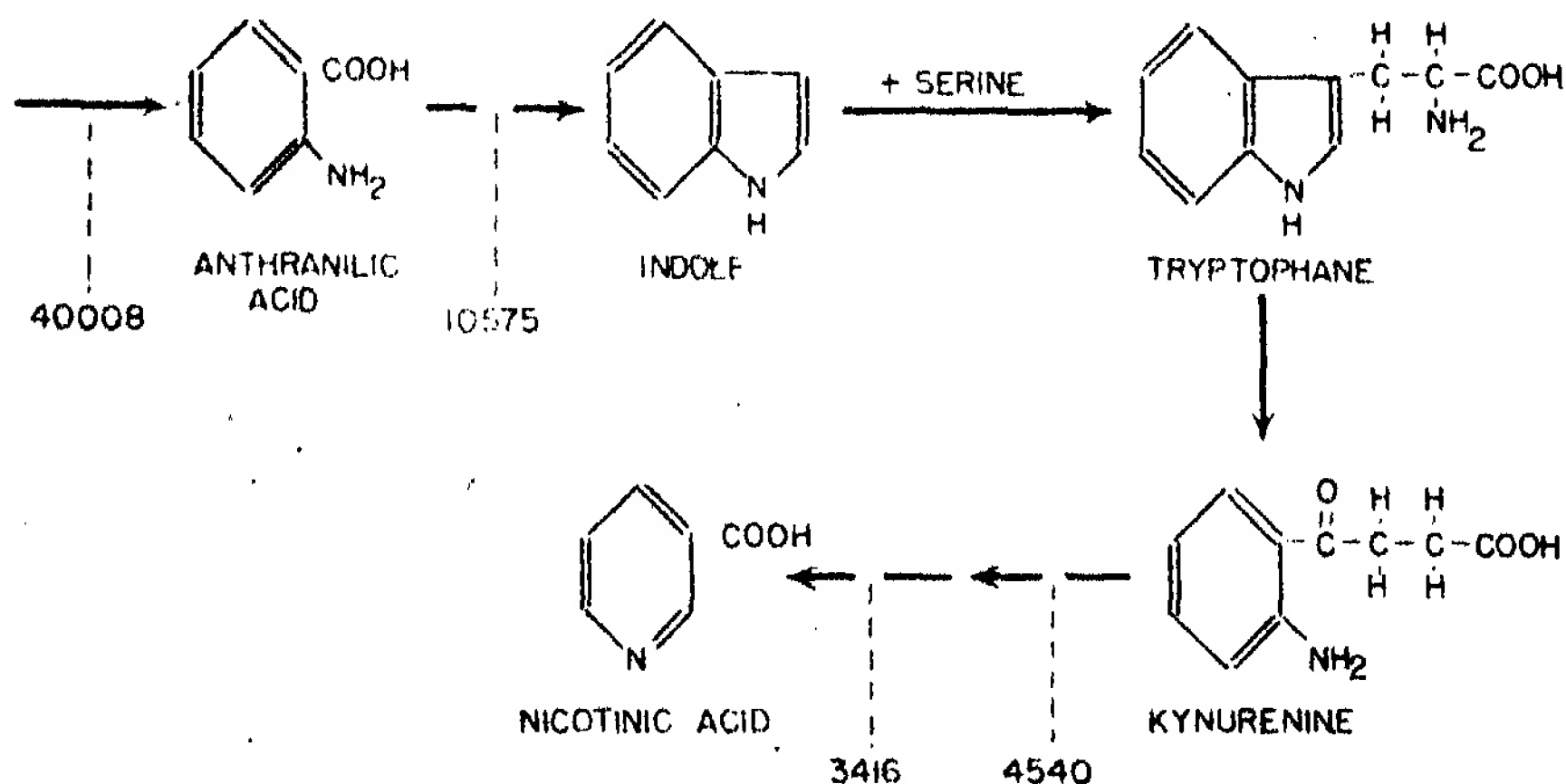


FIGURE 1

Proposed scheme of synthesis of tryptophane and nicotinic acid in *Neurospora*.

On the basis of the evidence so far available, we suggest that the synthesis of tryptophane and nicotinic acid in *Neurospora* takes place according to the scheme shown in figure 1. It is clear that this scheme is incomplete. The part played in it by the gene in which strain 65001 differs from wild type is not yet entirely clear but we suspect that it is concerned with one of the steps between anthranilic acid and indole. In addition the evidence strongly suggests that one of the functions of nicotinic acid is to act catalytically in the synthesis of tryptophane.

**Summary.**—On the basis of the growth responses of *Neurospora* mutant strains to tryptophane, nicotinic acid and kynurenine, it is postulated that nicotinic acid is normally formed from tryptophane with kynurenine as an intermediate. If this conclusion is correct, kynurenine has a biological significance not heretofore suspected. Whether kynurenine serves as an in-

intermediate between tryptophane and nicotinic acid in the mammal remains to be determined.

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## ON THE EVOLUTION OF THE GENUS *NICOTIANA*\*

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*Taxonomic Status.*—The relatively advanced family *Solanaceae* is allied to such families as the *Labiatae*, *Hydrophyllaceae*, *Boraginaceae* and *Polemoniaceae* but most closely related to the *Nolanaceae* and *Scrophulariaceae*. The approximately seventy-five genera have been organized in five to nine

tribes or subtribes. Don, Endlicher, Miers and Dunal placed *Nicotiana* in the tribe *Nicotianae*, Bentham and Hooker and von Wettstein, in the *Cestr(in)eeae*.

In taxonomic organizations of the *Solanaceae* the genera most frequently associated with *Nicotiana* are *Petunia*, *Cestrum*, *Sessia*, *Vestia*, *Metternichea*, *Retzia*, *Fabiana* and *Nierembergia*. From my point of view the morphological evidence more intimately relates the following genera to *Nicotiana*: *Petunia*, *Cestrum*, *Vestia* and *Fabiana*.

A recent taxonomic reorganization of the genus (Goodspeed<sup>1</sup>) adopts the treatment of Don and recognizes three subgenera and eleven sections with a total of fifty-eight species involved. In subgenus *Rustica* there are sections *Paniculatae* (seven species), *Thyrsoflorae* (one species) and *Rusticae* (one species); in subgenus *Tabacum*, sections *Tomentosae* (five species) and *Genuinae* (one species); in subgenus *Petunioides*, sections *Undulatae* (three species), *Trigonophyllae* (two species), *Alatae* (nine species), *Noctiflorae* (four species), *Acuminatae* (ten species) and *Suaveolentes* (fifteen species).

The significance with regard to evolutionary mechanisms in *Nicotiana* of certain of the inter- and intrageneric relationships defined in the taxonomic status of the genus briefly summarized above will appear in what follows.

*Morphology.*—*Nicotiana* is a conservative genus in the sense that it does not exhibit characteristic morphological specialization of particular floral or vegetative organs. On the other hand, no other genus of the family, save possibly *Solanum*, shows a greater range of variation in habit, inflorescence and flower than does *Nicotiana*, and the degree of physiological specialization attained by many of its species indicates the extent to which they have evolved.

Between the extremes represented, on the one hand, by desert ephemerals a few inches high and, on the other, by shrubby to subarborescent xerophytic or subtropical perennials to twenty-five feet in height are robust annuals some of which become limited perennials in favorable environments, and also root perennials spontaneously propagating from underground parts regardless of the fate of aerial ones. Inflorescence types include a thyrse panicle and a flat spray, between which complex extremes lie such intermediates and specializations as mono-, di- and pleiochasial cymes, solitary flowers and inconstant, diffuse paniculate-cymose mixtures.

Variability of the flower is chiefly expressed in the form and color of the corolla, stamen insertion and aestivation. In some species the flower is vespertine. The corolla is essentially salverform to trumpetform or tubular, the limb entire to rather deeply lobed. Red, green and yellow in various intensities and combinations occur. Pure white is found in only one species while a corolla predominantly white with a green to purplish flush

exteriorly is common. The stamens may be included or exserted, equal or unequal, long or short and inserted at any point from near the base of the corolla to immediately below the limb.

Consideration of major and minor details of habit, inflorescence and flower of *Nicotiana* species reveals correlations suggesting five definite but interrelated morphological nuclei or clusters of species. The *rusticoid* and *tomentosoid* nuclei can be related by shrubby habit, thyse inflorescence, non-vespertine corolla with tendency for the limb to pass slowly through an upright, horizontal and recurved condition, and low insertion of stamens. The other three—the *alatoid*, *noctifloroid* and *corymbosoid*—also show interrelation. They are herbaceous nuclei; the inflorescence is variable but not a thyse; the flower is usually whitish, vespertine and salverform; the limb seldom passes through a series of orientations; stamen insertion is higher, sometimes apical.

The *rusticoid* nucleus (subgenus *Rustica* in general, and a group of its member species in particular) shows greatest regularity in leaf form, size and distribution; evenness in distribution of branches; an inflorescence derivable from a leafy shoot and capable of producing other known inflorescence types in the genus by reduction; pronounced regularity of all four whorls of the flower; relatively little adnation of filaments; a conservative condition of corolla throat, stamens, pistil and hypogynous disc. On morphological grounds it is, therefore, taken to represent that portion of the genus in which the most primitive characters have been preserved. By contrast with various species of the *tomentosoid* and *corymbosoid* nuclei, the more strictly *rusticoid* members of subgenus *Rustica* exhibit relatively little polymorphy; the same is true of most members of the *alatoid* nucleus (including species of section *Alatae*) and less true of the *noctifloroid* (including species of section *Noctiflorae*).

The morphological evidence points to the rôle of hybridization in the origin of the five modern nuclei. Thus, one may assume that on a primitive ancestral level a series of progenitors of genera including pre*Nicotiana*, pre*Petunia*, pre*Cestrum* and the ultimate ancestors of other genera now related to *Nicotiana* began to vary each about a distinct morphological mean—differentiation referable to genic and structural alterations accompanied by combination and recombination, selection and genetic and geographic isolation. Two ancestral complexes, the *glauroid* and the *petunioid*, appeared as a result of hybridization of pre*Nicotiana* with, in the first case, pre*Cestrum* and, in the second, pre*Petunia*, followed by differentiation within two diverging modes of morphological variation thus established. Most of the members of the modern *rusticoid* and *tomentosoid* nuclei arose from the *glauroid* complex, while, to the same degree, the *alatoid*, *noctifloroid* and *corymbosoid* nuclei were derived from the *petunioid* complex. To this point and to this extent a dendroid diagram of the evolution of



*Nicotiana* might be constructed. However, certain species and species groups within and without the five nuclei cut sharply across such a diagram and transform it into a reticulum. Thus, there are modern species which owe their origin to hybridization between derivatives of the *glaucoïd* and *petunioid* complexes, and others, to hybridization between components of those ancestral complexes themselves.

✓ *Distribution.*— Today the distribution of the genus is essentially tripartite: South America, North America, Australia and the South Pacific. In South America *Nicotiana* occurs in approximately the entire "tail" of the continent, and also in southern and western Peru, the drier southwest of Ecuador, parts of Bolivia and the southern portion of western Brazil, but is absent from the drainage systems of the Amazon and Orinoco and from the northern countries of the continental "shoulder." In North America it is found native in central and northern Mexico and in the western United States. Scattered stations are known in Guatemala and the West Indies. In Australia it has been collected in all but the tropical north and northwest. In the South Pacific it extends in isolated localities from New Caledonia to the mid-Pacific Marquesas. This distributional picture is a product of the fact that, in general, modern species of *Nicotiana* demand light, a well-drained soil and dry rather than humid heat. Only a few species have set aside one or another of these requirements and occur on the margin of the moist tropics.

Presumably in South America existed the original reservoir from which the present-day assemblage of *Nicotiana* species arose. Certainly there is striking numerical preponderance of South American species and the extent of their differentiation into morphologically and cytogenetically distinct subdivisions has no parallel in the other two distributional areas. More specifically the center of distribution is taken to be the region now represented by the general area of junction of Peru, Bolivia, Argentina and Chile which corresponds to the current distribution of the *rusticoid* nucleus shown above to represent, on morphological grounds, the most primitive of the five clusters of modern species.

The tripartite, geographically discontinuous distribution of the genus is explicable on the assumption that ecologically suitable paths for migration connected South America with each of the other two continents in which species of *Nicotiana* are today native. The obvious suggestion that such a pathway to Australia included Antarctica and Tasmania is supported by the tracing of an extension of the Andean system into the Antarctic continent, the indication that a temperate flora existed there at a relatively recent time and that migration from Antarctica to Australia via Tasmania occurred. The problem of a route permitting passage from South to North America involves the semixerophytic nature of most modern *Nicotiana* species and the tropical zone which now separates the two



continents. There may have been a Caribbean path followed by certain progenitors more tolerant than current species of semitropical environments. Along it they could have passed from the northern "shoulder" of South America through the arc of the West Indies to Mexico, a route to which the distribution of other plants points more clearly. On the other hand, if the considerable decrease in temperature postulated for Central America during the glacial epoch induced a somewhat dry subtropical climate, migration might largely have followed the path of today's land connection between the two Americas. From central California to Mexico and from Chile to southern Peru a previously greater westerly extension of land has been postulated. Had a similar extension once existed between Mexico and Ecuador another pathway between central western South America and southwestern North America might have become continuous. In these various connections it is to be noted that both the western United States and Australia have undergone considerable desiccation since the presumptive advent of *Nicotiana*. In the former case this has encouraged the northerly spread of the genus; in the latter it has stranded, segregated and specialized forms already present. The South Pacific localities are considered (Wheeler<sup>2</sup>) to represent eastern migration from the Australian coast probably involving in the extreme instance—the Marquesas—introduction by man.

The distributional evidence indicates that the genus *Nicotiana* has maintained itself from at least a period prior to the formation of an effective Andean barrier, was present in Antarctica when that continent supported a temperate, herbaceous angiosperm flora, and passed from South to North America. It has survived land mass alterations of great magnitude which played a rôle in transforming a restricted, continuous area of distribution into a large, tricontinental and discontinuous one, and were responsible for forcing colonization of new areas and adaptation to new environments upon individual species, as well as for the extinction of others.

*Chromosome Number.*—In the *Solanaceae* haploid numbers from 7 to 36 have been reported; in the *Scrophulariaceae* the range is from 6 to 30. In the *Solanaceae* over fifty-five per cent of the species investigated show 12 pairs while a multiple of 12 pairs occurs in an additional twenty per cent. In the *Nolanaceae* all the five species counted have 12 pairs and in the *Scrophulariaceae* species with 12 as well as species with 6 pairs occur.

Of the fifty-eight species of *Nicotiana* the chromosome numbers of fifty-five are known. Twenty-eight species show 12 pairs; eleven, 24; four, 16; three, 9; two, 10; two, 19; two, 20; in one species each the haploid numbers are 18, 21 and 22. In related genera the known haploid numbers are: 7 (*Petunia*), 8 (*Cestrum*, *Vestia*), 9 (*Petunia*, *Fabiana*, *Nierembergia*), 22 (*Salpiglossis*).

Subgenus *Rustica* and subgenus *Tabacum* contain only species with 12 or 24 pairs. In the third subgenus (*Petunioides*) four of the six sections contain species showing 24 pairs and all but one section species showing 12 pairs. Two aneuploid series occur, each in a separate section of subgenus *Petunioides*: 9 to 12 pairs in section *Alatae* and 16 to 24 pairs in section *Suaveolentes*.

The rôle of hybridization in *Nicotiana* phylogeny suggested by the morphological evidence offers a partial explanation of the origin and evolution of the present-day chromosome number situation just summarized. The primitive ancestral reservoir above referred to which included at least pre-*Nicotiana*, pre-*Cestrum* and pre-*Petunia*, and which via hybridization on the pregeneric level gave rise to the basic *Nicotiana* complexes, the *glauroid* and the *petunioid*, is assumed to have been 6-paired. From the two 6-paired complexes a 12-paired *Nicotiana* level arose by interspecific hybridization followed by chromosome doubling and, to a lesser extent, by autopolyploidy. During the establishment of the 12-paired level a residue of 6-paired species, now extinct, was also in existence. Finally, from the 12-paired level, an almost exclusively allopolyploid 24-paired level was developed, thus contributing largely to the transformation of a basically dendroid into a reticulate diagram of evolution of *Nicotiana*.

Two aneuploid sequences arose, one between the 6- and 12-paired levels, the other below the 24-paired level. The former is today represented only by 9- and 10-paired species, all of them in section *Alatae*, although earlier existence of 8-paired species may be reflected, as will be indicated below, in the occurrence of the other (16- to 24-paired) aneuploid series. In this connection it is to be recalled that 7-, 8-, 9- and 22- ( $x = 11$ ) paired species are found in modern genera related to *Nicotiana*.

The postulated lower numbers in the 6- to 12-paired aneuploid sequence presumably had a sesquidiploid origin. The higher numbers were doubtless derived by reduction from the 12-paired condition, perhaps in part involving reciprocal translocation (cf. Babcock<sup>3</sup>). There are a number of explanations of the origin of the 16- to 24-paired aneuploid series, all species of which are members of the section *Suaveolentes*. In my opinion two or more 24-paired ancestors of section *Suaveolentes* arose, as did the nine modern 24-paired species, by amphiploidy on the early 12-paired level. During a corresponding period, the same or related 12-paired species, crossing with certain of the not yet extinct 6-paired species, produced by chromosome doubling the 18-paired condition which by reduction initiated the 16-paired group of species. Such establishment of the 16-, 18- and 24-paired progenitors of section *Suaveolentes* was followed by the production of 19-, 20-, 21- and 22-paired species as a result of hybridization, sesquidiploidy, addition through fragmentation or reduction by loss or fusion. Other suggested explanations (Kostoff<sup>4</sup>) involve now extinct 8-, 9- and 10-

paired species in the amphiploid production of 16-, 18- and 20-paired species or take 16- and 20-paired species to be secondary derivatives from an 18-paired one so derived. Yet another (Wheeler<sup>5</sup>) assumes the former existence of a series of 8-paired species which by successive amphiploidy gave rise to the 16- and then the 24-paired condition, with subsequent hybridization between the products resulting in the 18- to 22-paired sequence. In any case, it appears that this modern aneuploid sequence represents a series of secondary derivatives with amphiploidy the fundamental mechanism responsible for their origin.

*Chromosome Morphology.*—For the genus as a whole a combination of median (*m*) and submedian (*sm*) chromosomes predominates over subterminal (*st*) ones in the ratio of five to three. In each of the three subgenera there is a distinctive ratio of *m* plus *sm* to *st*: in subgenus *Rustica* it is 9:1, in subgenus *Tabacum* 5:2 and in subgenus *Petunioides* 4:3. In addition, there is a basic karyotype characteristic of those species which represent the morphological core of each section. In other words, chromosome morphology in *Nicotiana* exhibits sufficiently distinctive features to have phylogenetic significance (Goodspeed<sup>6</sup>).

In the species of genera related to *Nicotiana* for which chromosome morphology is known, the genomes consist almost exclusively of *m* or *sm* chromosomes. Thus, in species investigated of both *Cestrum* and *Vestia* all of the chromosomes are *m* or approximately *m*, and in 7-paired species of *Petunia* 5 are *m*, 1 is *sm* and 1 is *st*.

The contention that primitive groups tend to possess predominantly *m* chromosomes while those of more advanced status show an increasing proportion of heterobrachial ones is apparently borne out in *Nicotiana*. Based upon this contention, the above postulated aggregation of 6-paired ancestral species probably possessed all *m* chromosomes. Subsequent evolutionary levels, progressive in terms of chromosome multiplication and with aneuploidy as their by-products, were in most instances characterized by differentiation in chromosome morphology, particularly at the 12-paired level. Thus, although the *rusticoid* derivatives (species of subgenus *Rustica*) of the *glaucoïd* complex have preserved almost exclusively the primitive *m* type of chromosome, its *tomentosoid* derivatives (species of subgenus *Tabacum*), derivatives of the *petunioid* complex (species of subgenus *Petunioides*) and species derived by hybridization between the two complexes themselves today combine *m*, *sm* and *st* chromosomes in varying proportions. In the *rusticoid* nucleus the tendency toward preservation of the postulated ancestral *m* type of chromosome may be a product of the initial setting apart of a relatively more uniform, and thus more stable, type of internal chromosomal structure, perhaps genically conditioned, together with the persistence of relatively uniform or favorable environmental conditions.

There is evidence suggesting the direction which possible future alteration of the typical *m* karyotype of subgenus *Rustica* may follow. In all 12-paired species of *Nicotiana* at least four nucleoli are seen, and apart from those of subgenus *Rustica* the majority of the species correspondingly show four satellites. In seven of the eight 12-paired species of subgenus *Rustica* only two satellites appear. However, in each of them at least one *sm* chromosome consistently shows a distinct tertiary constriction which is presumably associated with a nucleolus organizer. Such chromosomes, by fragmentation at the distal portion of the less stable heterochromatic regions doubtless associated with these tertiary constrictions, might be expected to become transformed into *st* satellited chromosomes. As a product of an equivalent internal structural differentiation followed by fragmentation the full complement of satellited chromosomes characteristic of the majority of the species of subgenus *Tabacum* and subgenus *Petunioides* could have been derived. The transitional position in terms of satellite evolution which subgenus *Rustica* apparently occupies would argue in favor of its primitive status, one which is indicated on other grounds.

*Meiotic Chromosome Behavior.*—Complete pairing between structurally homologous chromosomes is shown at *MI* in the majority of species. In certain 12-paired species, however, bridges and fragments at *AI*, and in others frequent occurrence of non-conjunction or non-disjunction followed by irregular distribution of chromosomes, give evidence of the structural hybridity of their genomes. In only one species, an amphiploid on the 24-paired level, has the presence of multivalents been observed. Study of meiotic behavior in races of at least one polymorphic species demonstrates that certain of these races differ from one another by one to several reciprocal translocations and suggests the rôle of structural alterations in initiating species differentiation in *Nicotiana*.

All of the twenty-four monosomics of *N. tabacum* (Clausen and Cameron<sup>7</sup>) and all of the twelve primary trisomics of *N. sylvestris* (Goodspeed and Avery<sup>8</sup>), as well as numerous other chromosomal variants involving loss or addition of one to several chromosomes have been studied in respect to meiotic chromosome behavior. These and similar investigation of variants involving reduction or multiplication of entire genomes—haploids, triploids and tetraploids—have yielded evidence of evolutionary significance. For example, the conspicuous morphological distinctions characterizing each of the primary trisomics of a single species demonstrate the extent to which such chromosome unbalance may be reflected in differences in morphological expression. For example, again, the occurrence at *MI* in tetraploid forms of a number of 12-paired species of valencies higher than four (Fardy and Hitier<sup>9</sup>) suggests the compound character of the diploid representatives of those races.

Meiotic chromosome behavior at *MI* has been studied in a total of two



hundred and thirteen  $F_1$  interspecific hybrids of *Nicotiana*. While this number of hybrids represents but a small percentage of the possible combinations of the fifty-eight species of the genus, they include representative combinations pertinent for interpretation of relationships: fifty-three species are involved in the eighty-three intrasectional, forty-nine inter-sectional and eighty-one intersubgeneric hybrids analyzed.

On the basis of the results of these  $MI$  analyses the two hundred and thirteen  $F_1$  interspecific hybrids may be classified into five categories: lack of pairing, complete, low variable, high variable and "Drosera Scheme" pairing (cf. Goodspeed<sup>10</sup>). In the high and low variable categories where an extremely wide range in number of pairs may occur the high point in the range is considered significant as indicating the maximum number of appreciable homologous segments in the two genomes involved, while the mode may be an expression of normal expectancy on the basis of the maximum number of homologous segments present or may indicate the number of chromosome pairs possessing relatively large pairing blocks. Similarly, in the lack of pairing hybrids with a range of zero to 4 (frequently 0 to 1, 2 or 3), mode zero, the occurrence of up to 4 pairs presumably represents the maximum, though not consistent, expression of small homologous segments residual in the genomes involved, while the characteristic mode of zero is largely a reflection of the lack of pairing blocks of appreciable size and effectiveness. The presence of a limited number of non-homologous segments in the genomes of the two parental species may explain the occasional occurrence of univalents in a hybrid in which the mode is complete pairing. Therefore, on the basic assumption of the significance of pairing (cf. Stebbins<sup>11</sup>), the maximum extent of pairing in an  $F_1$  interspecific hybrid may be considered as an index of the relationship of the species involved in terms of their residua of ancestral homology. Such application of extent of pairing as a criterion of relationship in *Nicotiana* largely confirms the morphological evidence expressed in the taxonomic organization of the genus. Thus, in approximately ninety per cent of the  $F_1$  hybrids investigated amount of pairing parallels extent of taxonomic relationship of the parental species. In the ten per cent in which extent of pairing does not correspond to degree of taxonomic relationship some few show higher, while the majority show lower, than the amount of pairing to be anticipated. Exceptions of the former type furnish an illustration of the value of a pairing check on a taxonomic organization. Although genetic alterations, reflected in morphological distinctions setting apart two species, have occurred, genically they have more in common than other species of a similar degree of morphological differentiation which show negligible pairing in the  $F_1$  hybrid between them. This means that, in the one case as contrasted with the other, there has been less fundamental divergence from a common (or related) ancestor. On the other hand, lower pairing in  $F_1$  than

corresponds to degree of taxonomic relationship between the two parental species may be attributed to one or more of the factors recognized as contributing to secondary disturbance of conjugation.

In terms of hybrids which reach maturity and thus are available for cytological analysis *Nicotiana* is apparently exceptional, at least among genera extensively investigated, in degree of compatibility between its species. Thus, relative remoteness in relationship is not a bar to compatibility, as indicated, first by the fact that approximately sixty-five per cent of  $F_1$  interspecific hybrids show meiotic irregularities, and second, as noted above, that the same proportion involve as parents members of different subgenera or sections. In other words, it appears that factors restricting compatibility have evolved less rapidly than those inhibiting pairing and those responsible for specific morphological distinctions. Certainly in *Nicotiana* high degree of compatibility is characteristic of many present-day species which have diverged considerably in respect both to external morphology and to extent of pairing in  $F_1$  hybrids between them.

In some cases both, and in all cases one, of the species related to the 12-paired ancestry of each of the nine modern American species on the 24-paired level can be identified by meiotic chromosome behavior in appropriate  $F_1$  interspecific hybrids. For three of the nine an amphiploid origin is recognized, with identification of the present-day 12-paired descendants of both the original parental races provided in large part by the "Drosera Scheme" pairing relations exhibited by the hybrids between these descendants and the 24-paired species in question. Of these three, *N. arentsii* furnishes a demonstration of an evolutionary sequence initiated by amphiploidy which is today in progress. Thus, the descendants—*N. undulata* and *N. wigandioides*—of the two 12-paired races which entered into its amphiploid origin possess in their genomes sufficient homologous segments to produce a considerable number of bivalents at  $MI$  of the  $F_1$  hybrid between them. Therefore, the original parents represented only certain extents of differentiation, both genic and structural, from a common ancestor or closely related ancestors on the 12-paired level. Consistent with this proposition is the occurrence in *N. arentsii* of multivalents which also appear frequently at  $MI$  in  $F_1$  hybrids between that species and *N. undulata* and *N. wigandioides*. By contrast, *N. tabacum*, another 24-paired species of known amphiploid origin, combines in its ancestry progenitors of two 12-paired species—*N. sylvestris* and *N. otophora* or another member of the same section—which are today taxonomically remote. Correspondingly, the  $F_1$  hybrid between these two 12-paired species shows almost complete lack of pairing and no multivalents appear at  $MI$  in *N. tabacum*. This evidence indicates that the considerable degree of multivalency exhibited by  $F_1$  hybrids between *N. tabacum* and both *N. sylvestris* and *N. otophora* (or its relatives) is referable to translocations within the genomes



of *N. sylvestris* and of *N. otophora* or within the genom of *N. tabacum* during the evolution of these three species.

For the remaining six 24-paired American species an amphiploid origin is indicated by "Drosera Scheme" pairing or approximation thereof at *MI* in *F*<sub>1</sub> hybrids between each of these species and a specific 12-paired species. In each case the modern descendants of the other original parent on the 12-paired level have presumably become extinct. However, in some instances morphological and in others cytogenetic evidence points to the position in the author's taxonomic organization of the genus which those species would have occupied. Both types of evidence suggest for certain pairs of modern 24-paired species one common 12-paired ancestor, in some instances represented by descendants today, in others not so represented.

The group of related species including *N. repanda*, *N. nesophila* and *N. stocktonii* provides a present-day illustration of species differentiation from a common ancestor on the 24-paired level. Morphologically the distinctions within the group pattern are considerable but complete pairing in *F*<sub>1</sub> intragroup hybrids occurs. The case of *N. arentsii*, discussed above, provides a possible picture of the origin of such species groups since the products and by-products of multivalent formation, including substitution between members of subgenoms, to be anticipated in the evolution of *N. arentsii* could initiate species differentiation.

Processes similar to those known to be responsible for origin of the 24-paired level from progenitors of known 12-paired species were presumably operative in initiating the 12-paired level from postulated 6-paired ancestral species (cf. Kostoff<sup>4</sup>). Parallel origin from a primitive 6-paired ancestral level not only of pre*Nicotiana* but also of, at least, pre*Cestrum* and pre*Petunia* permitted inter-pregeneric hybridization. In part the product of such hybridization on the next level of differentiation, when those various genera were establishing their distinct modes of morphological variation, was, as already noted, the definition of the 6-paired *glaucoïd* and *petunioid* complexes in *Nicotiana*. A subsequent period of expansion of these two complexes gave a series of species whose ranges of morphological variation in some instances did and in others did not appreciably overlap. Correspondingly, their genomes became to different extents mutually exclusive in terms of genic content and structural organization.

Crossing between 6-paired races of various degrees of differentiation from the pre*Nicotiana* level onward, followed by chromosome doubling, was doubtless a determining factor in the origin of the 12-paired level, just as such amphiploidy has been largely responsible for the origin of the modern 24-paired level. On this assumption, the extent of fundamental relationship between 6-paired ancestors plus accumulation of genic and structural distinctions since establishment of the 12-paired level are reflected in the amount and character of pairing at *MI* of *F*<sub>1</sub> hybrids be-

tween present-day 12-paired species! Thus, complete pairing in such hybrids may be interpreted as the expression of an originally maximum degree of relationship together with minimum later accumulation of genic and structural alterations. The opposite extreme, almost complete lack of pairing, would then be referable to minimum initial relationship plus maximum incidence of distinctions subsequently produced, while low and high variable pairing indicate intermediate expressions of the two factors.

Obviously, the possibility of an auto- as contrasted with an essentially allopolyploid origin of certain members of the original 12-paired from the 6-paired level cannot be excluded. The occurrence of multivalency in current 12-paired species or evidence of autosyndesis in  $F_1$  hybrids between them should follow such origin. The first of these consequences has not, in my experience, been demonstrated and evidence concerning the second would be difficult to obtain because of lack of large morphological distinctions at  $M_1$  between members of the genomes of *Nicotiana* species. On the other hand, it is possible that autosyndesis, a product of autopolyploid origin of one or both parents, may explain in certain instances high variable pairing in  $F_1$  hybrids between modern 12-paired species. A 6-paired ancestor in common between two such species, each originating by allopolyploidy, could be reflected in low variable pairing. In any case, pairing variability is doubtless a product largely of differentiation after attainment of the 12-paired level which has served to reduce the size and alter the arrangement of originally extensive homologous segments.

*Conclusion.*—The point of view presented here concerning the evolution of *Nicotiana* emphasizes the rôle which hybridization has played in establishing the modern expression of the genus, assuming the existence of a now extinct reservoir of 6-paired pre*Nicotiana* races from which the progenitors of modern 12-paired and 24-paired species levels were derived, largely by amphiploidy. Differentiation on the 6-paired level produced two basic ancestral complexes in the development of which hybridization between pre*Nicotiana* and at least pre*Cestrum* and pre*Petunia* was concerned. From these complexes was produced a series of 12-paired derivatives certain of which are united in the subdivisions of the author's taxonomic treatment of the genus while others trace their origin more directly to both the ancestral complexes via hybridization between their components or immediate derivatives. The present-day 24-paired species level arose largely by amphiploidy involving members of most of the 12-paired clusters of species into which the ancestral complexes became segregated. Throughout the evolutionary sequence genic and structural chromosomal alterations played a part at first more, and later less, definitive than amphiploidy in producing differentiation at successive levels, culminating in the range of morphological variation exhibited by the current *Nicotiana* species assemblage. Documentation of these propositions is afforded by the combined taxonomic,

morphological, distributional and cytological evidence, the most pertinent aspects of which have been briefly outlined above.

In evaluating the present and predicting the future evolutionary status of *Nicotiana* it is to be borne in mind that evidence indicating antiquity does not necessarily indicate senescence from the genetic point of view. Although the extent of morphological, and especially physiological, specialization attained by many species of *Nicotiana* suggests a point in the generic cycle past maturity, the degree of polymorphy indicates a plasticity and capacity for expansion characteristic of a relatively aggressive stage of the cycle (Clausen, Keck and Hiesey<sup>12</sup>). The degree of polyploidy already attained by the genus, a product of the continuing and definitive rôle of hybridization in its evolutionary history, might argue for its advanced or even senescent status. On the other hand, it appears that hybridization with the genom or the gene as the unit of differentiation remains a potentially effective mode of future evolution because apparently genetic barriers are accumulating less rapidly than genic alterations determining species distinctions, with the result that there is a high degree of intersubgeneric as well as intersectional and intrasectional crossability. While the present-day range of distribution of the genus in terms of species is, in general, locally restricted and apparently not increasing, the tripartite nature of its global spread argues against the possibility of extinction since, obviously, future climatic or other alterations would be unlikely to be operative over a sufficient geographic area to eliminate all its species. Indeed, on the assumption that the early part of an interglacial period now obtains, the future extension of a genus, most species of which are arid or semiarid types, would be favored by increasingly warmer, drier climates. In other words, despite the fact that numbers of species of *Nicotiana* have doubtless become extinct, that others have become extremely restricted in extent, and that many are today polyploids exhibiting considerable specialization, survival of *Nicotiana* as a genus is favored by its relatively wide range in distribution and morphological character together with its lack of barriers to hybridization. ✕

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## ANTIBIOTIC SUBSTANCES FROM BASIDIOMYCETES I. *Pleurotus griseus*\*

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In a survey<sup>1</sup> conducted in this laboratory it was noted that 213 of the 332 species of Basidiomycetes studied produced substances that inhibited the growth of *Staphylococcus aureus*. Among the species that seemed worthy of further investigation was *Pleurotus griseus*.<sup>2</sup>

*P. griseus* grew with medium rapidity on a thiamine-peptone agar<sup>1</sup> and a modified Czapek-Dox agar to which corn steep solids had been added. It formed a pink pigment which diffused into the agar. After the fungus had grown for one to several weeks, discs 5.5 mm. in diameter were cut on a radius extending from the center of the colony into the agar adjacent to the colony. These discs were placed on a yeast-peptone agar seeded with *Staph. aureus* and the plates incubated overnight at  $37 \pm 2^\circ\text{C}$ . Clear zones of inhibition were found around the discs indicating that some substance or substances had diffused from the agar discs into the surrounding seeded medium in sufficient concentration to inhibit the growth of the staphylococci in the agar. A disc cut from the agar at as great a distance as 20 mm. from the edge of the colony frequently produced a zone of inhibition. The zones, while small, were clear, indicating that bacteria resistant to the antibiotic substance or substances were absent. Disc tests, with strains of *Staph. aureus* resistant to other antibiotic substances, indicated that *P. griseus* produced an antibiotic substance which was different from penicillin and different from the active substances produced by several other Basidiomycetes. The antibiotic substance or substances from *P. griseus* did not inhibit the growth of *Escherichia coli*.

*P. griseus* was grown on several types of nutrient media, differing from one another in the source of nitrogen and carbon, to determine the effect of the medium on the production of the antibacterial substances by the fungus. Cane sugar, dark brown sugar, galactose, lactose, mannitol and corn steep solids were ineffective carbon sources; maltose and soluble starch were poor; dextrose and levulose were the best and nearly equal so far as the



production of antibacterial substances was concerned. Various amounts of dextrose ranging from 10 to 100 g. per l. were tested; 40 g. per l. seemed somewhat superior to 20 or 60 g. Nitrates and asparagine in the presence of dextrose, were unsatisfactory sources of nitrogen; corn steep solids, an extract of corn steep solids made with 90 per cent methanol, N-Z-CASE, amigen and neopeptone were all effective. On the basis of this survey it was decided that a practical medium to use for liquid culture was a modified Czapek-Dox mineral solution<sup>3</sup> to which 40 g. of dextrose and 5 g. of corn steep solids were added per liter of solution.

*Antibiotic Material in Liquid Culture.*—The fungus was grown at 25°C. in Fernbach flasks on coils of beech shavings, using one liter of the above medium per flask. After a growth period of about one month, the fungous mat had nearly covered the entire liquid surface; the liquid had an activity of about 1000 dilution units per ml. when assayed with *Staph. aureus* (Heatley strain). The culture fluid was then decanted and replaced by one liter of fresh corn steep medium. After a further incubation period of from 5 to 7 days, the antibacterial activity of the culture fluid in the reflooded flask was usually at least 256 dilution units. This culture fluid was then decanted and replaced by fresh nutrient solution. This reflood technique saved from seven to fifteen days in the production of each lot of active culture fluid and was especially useful for those Basidiomycetes which grow slowly.<sup>4</sup> Flasks which had been reflooded nine times have shown as rapid and as great a production of antibacterial substance during the last period of reflooding as during the first.

*Isolation of an Antibiotic Substance.*—Pooled culture fluids with an activity of from 256 to 1024 dilution units per ml. were strained through cheesecloth to remove bits of mycelium and wood. They were then extracted by shaking with a one-tenth volume of chloroform. The chloroform-in-water emulsion was removed and centrifuged to break the emulsion. The chloroform portion was extracted three times with one-tenth volume of one per cent sodium bicarbonate solution to remove acids. The chloroform solution was then reduced to a few milliliters by distillation under reduced pressure, transferred to a small beaker and left at room temperature until all the chloroform had evaporated. A reddish gum in the beaker was dissolved in the minimum volume of hot ethanol, ether was added, the beaker covered and let stand at room temperature to permit slow evaporation of the solvents. Orange-colored crystals formed soon after the ether was added. The crystals were removed, washed with ether and air-dried. The product was recrystallized from a chloroform-ether solution. Large amber colored crystals were formed when evaporation of the solvents took place very slowly. Fine needle-like yellow crystals were formed as a result of rapid evaporation of the solvents. From 100 to 180 mg. of the crystalline material were obtained per l. of culture fluid.

A solution prepared from the crystals showed activity after 24 hrs. against *Staph. aureus* at a concentration of 1  $\mu$ g. per ml. of solution and no activity against *E. coli*. The crystalline antibiotic substance was named pleurotin.

*Chemical Properties of Pleurotin.*—Pleurotin began to melt with decomposition at temperatures between 200° and 215°C. depending upon the rate of heating. Qualitative tests indicated the absence of ash and of the following elements: halogens, nitrogen and sulfur.

Microanalysis<sup>5</sup> gave the following: C, 70.83, H, 6.43; Mol. wt. (Rast) 343; MeO, 0.0. The computed values for an empirical formula of  $C_{20}H_{22}O_5$  were C, 70.16; H, 6.47; Mol. wt. 342.4. Pleurotin was optically active with  $\alpha_D^{23} = -20^\circ$ ;  $C = 0.59$  m. chloroform. We are indebted to Dr. J. D. Dutcher of The Squibb Institute for Medical Research for the molecular weight, analysis and optical rotation.

The absorption spectrum had a single absorption peak in the ultra violet at 2500 A for which the molecular extinction co-efficient was 13,680 (molecular weight assumed to be 342) for a solution in 4 per cent ethanol. The absorption in the visible was not measured.

The solubility of pleurotin at 25° was in water 0.125 mg. per ml., in 95% ethanol 6.8 mg. per ml., in 5% ethanol, 0.37 mg. per ml., in ether 3.5 mg. per ml. and in chloroform more than 200 mg. per ml. It was relatively insoluble in dilute acids, dilute solutions of sodium bicarbonate and in petroleum ether. It was more soluble in acetone.

Pleurotin did not give a color reaction with ferric chloride. It liberated iodine from acidified potassium iodide solution. Its reaction with a solution of potassium cyanide to give a blue color was used as the basis of a colorimetric method suitable for the quantitative determination of pleurotin in culture fluids and other solutions.

Pleurotin was adsorbed from culture fluids by Norit A and eluted from the air-dry carbon by chloroform. Pleurotin is a neutral substance which reacts with alkali to give an acidic product devoid of antibacterial activity.

Pleurotin was not thermostable. Solutions of pleurotin in 0.1 M. phosphate buffer when boiled for ten minutes lost 50 per cent of their biological activity at pH 3, 75 per cent at pH 6.5 and all their activity at pH 8.5 and higher. Pleurotin was 75 per cent destroyed in one hour at pH 8.5 and 25°C. An aqueous solution containing 100  $\mu$ g. of pleurotin per ml. lost 30 per cent of its pleurotin as determined chemically after autoclaving at 120°C. for 15 min. Pleurotin in solution was rendered inactive by exposure to light for a few hours. It was filterable through a Seitz filter pad.

The chemical and physical properties of pleurotin are sufficient to establish it as different from all other antibiotic substances that have been prepared in pure form. The low solubility of pleurotin in aqueous solution and its instability suggest that its possible therapeutic value is unlikely.



**Antibacterial Action of Pleurotin.**—The antibacterial activity of pleurotin was determined by the methods in use in this laboratory.<sup>6</sup> The bacteria used were: *Staph. aureus*, *Klebsiella pneumoniae*, *Photobacterium fischeri*, and the following standard tester strains of S. A. Waksman: *E. coli*, *B. mycoides* and *B. subtilis*. The following table gives the minimum antibacterial concentrations of pleurotin after an incubation period of 24 hours:

BACTERIA	μG PER ML.
<i>Staphylococcus aureus</i>	0.8
<i>Bacillus mycoides</i>	1.6
<i>Bacillus subtilis</i>	0.2
<i>Escherichia coli</i>	500.0
<i>Klebsiella pneumoniae</i>	500.0
<i>Photobacterium fischeri</i>	6.0

The activity for incubation periods of 16 to 18 hrs. was somewhat higher and for 48 hrs. was one-half or one-quarter the values given.

Pleurotin was active only on the gram-positive bacteria. The culture fluid from *Pleurotus griseus* was active only on the gram-positive bacteria indicating the absence of an appreciable amount of a second antibiotic substance with antibacterial properties markedly different from those of pleurotin. The antibacterial activity of a culture fluid was equal to that of a solution of pure pleurotin of the same concentration, the pleurotin content of the culture fluid being determined by the potassium cyanide method.

**Toxicity for Mice.**—Tests made by Dr. G. Rake at the Squibb Institute for Medical Research indicated that a single dose of pleurotin was not toxic when given intravenously to white mice at the rate of 24 mg. per kilogram of body weight. Pleurotin is so insoluble in aqueous solutions that larger amounts could not be given. For the twenty studies 11.4 mg. of pleurotin were dissolved in 1.0 ml. of warm ethanol and diluted with 19.0 ml. of warm saline solution. By maintaining the solution at 37° crystallization was delayed long enough to permit intravenous injection.

**Activity on *M. tuberculosis*.**—Through the courtesy of Dr. Ralph R. Mellon, Institute of Pathology, Western Pennsylvania Hospital, Pittsburgh, Pa., tests were carried out by Miss Jean Onslow on *Mycobacterium tuberculosis*. Discs cut from a 29-day old colony of *P. griseus* grown on the modified Czapek-Dox medium containing corn steep solids showed inhibition of an avirulent strain of *M. tuberculosis* H 234 on a yeast peptone agar. The activity was confined to the discs from the colony and was less than for *Staph. aureus*. A comparison for the two organisms is given in the following table where the inhibition in mm. is given for 6 discs taken on a radius extending from the center of the colony. Discs 1 and 2 came from the fungous colony.

ORGANISM \ DISC						
	1	2	3	4	5	6
<i>Staph. aureus</i>	17	17	11	9	8	Halo
<i>M. tuberculosis</i> H 234	11	7	0	0	0	0

Agar discs saturated with pleurotin produced no zones of inhibition. Pleurotin at a concentration of 100  $\mu$ g. per ml. of Youmans' medium<sup>7</sup> or Kirchner's medium<sup>8</sup> was ineffective.

Discs from a colony *P. griseus* 9 days old were tested on virulent *M. tuberculosis* H 37 grown on Herrold egg medium. A disc taken from within the limits of the fungous colony produced an inhibition zone of 7 mm. Discs from the edge of the colony or in the agar adjacent to the colony were ineffective.

*Relative Activity of Pleurotus griseus and pleurotin.*—Some observations made during the course of our investigation indicated that all of the antibacterial activity of *P. griseus* was not accounted for by pleurotin. Disc cut from within the limits of a colony of *P. griseus* gave zones of inhibition on *Staph. aureus* and *M. tuberculosis* considerably larger than those obtained with similar discs saturated with pleurotin. The presence in the mycelium of an enzyme capable of forming an antibacterial substance during the incubation of the discs at 37° or of a factor which enhanced the activity of pleurotin was not demonstrated by our experiments.

On the other hand, an aqueous extract of the mycelium tested by the cup method against *Staph. aureus* was considerably more effective than a saturated solution of pleurotin and an acid fraction.<sup>9</sup> prepared from culture liquid gave large zones of inhibition.

The acid fraction showed some activity (1333 dilution units per g.) on *M. tuberculosis* H 234, although a saturated solution of pleurotin was inactive. The activity of this fraction on *Staph. aureus* was 8000 dilution units per g.

This evidence was taken to indicate the production by *P. griseus* of an antibacterial substance or substances other than pleurotin. Since the unidentified substances were not isolated, we do not know whether they fall within the antibiotic range (effective at a dilution of 1 to 40,000 or less). In any event, the quantity in the culture liquid must be small since within the limits of error the antibacterial activity of the culture liquid can be accounted for on the basis of its pleurotin content as determined chemically.

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<sup>7</sup> Robbins, W. J., Hervey, A., Davidson, R. W., Ma, R., and Robbins, W. C., *Bull. Torrey Bot. Club*, 72, 165-190 (1945).

<sup>8</sup> The fungus was obtained from Dr. Ross W. Davidson and is numbered 14616-R in his collection. Another isolation of *Pleurotus griseus* obtained from Dr. Mildred K. Nobles was also active. No difference in the pleurotin formed by the two strains was detected.

<sup>3</sup> The mineral solution contained per liter, 3 g.  $\text{NaNO}_3$ , 1 g.  $\text{KH}_2\text{PO}_4$ , 0.5 g.  $\text{KCl}$ , 0.5 g.  $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$  and 0.01 g.  $\text{Fe}_2(\text{SO}_4)_3$ .

<sup>4</sup> Robbins, W. J., Kavanagh, F. A., Hervey, A., *Ann. N. Y. Acad. Sci.*, **48**, 67-72 (1946).

<sup>5</sup> The microanalyses and molecular weight determinations were carried out by Mr. J. J. Alicino of The Squibb Institute for Medical Research.

<sup>6</sup> Kavanagh, F., *Bull. Torrey Club*, **74** [in press].

<sup>7</sup> Youmans, G. P., *Proc. Soc. Exp. Biol. and Med.*, **57**, 119-122 (1944).

<sup>8</sup> Kirchner, O., *Zbl. f. Bakt. I Orig.*, **124**, 403-412 (1932).

<sup>9</sup> The acids removed from the chloroform extract of the culture liquid by treatment with  $\text{NaHCO}_3$ , as described previously, were dissolved in ether. The ether solution was extracted with 1 per cent  $\text{NaHCO}_3$ , the bicarbonate solution acidified and the organic acids passed into ether. This process was repeated nine times, a procedure which should have removed all pleurotin. The acid fraction obtained in this way was taken to dryness and extracted with water. The resulting water extract was the acid fraction referred to in the text.

## ANTIBIOTICS FROM BASIDIOMYCETES II. *Polyporus biformis*\*

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In a survey of fungi reported earlier,<sup>1</sup> *Polyporus biformis*<sup>2</sup> was found to produce antibacterial substances. Its activity on *Staphylococcus aureus* (H) as evidenced by the disc test, encouraged us to investigate it further.

This fungus grew well at 25°C. on malt agar, thiamine-peptone agar and a modified Czapek-Dox agar to which corn steep solids had been added,<sup>1</sup> and produced antibacterial substances on all three media. Tested by the streak method, inhibition zones extending 12 to 25 mm. from the edge of the fungus colony were observed. The inhibition of *E. coli* was less than that of *Staph. aureus*. When the antibacterial activity was tested by the agar disc<sup>1</sup> method, inhibition areas as large as 25 mm. in diameter with a 5.5 mm. disc were obtained with *Staph. aureus*.

Culture liquids of *P. biformis* evidenced antibacterial activity. The fungus was grown at 25°C. in 2800 ml. Fernbach flasks containing 1 l. of modified Czapek-Dox medium with dextrose and corn steep solids<sup>3</sup> on coils of beech wood shavings which furnished mechanical support for the mycelium. After a growth period of 2 weeks or more, the activity of the culture liquid on *Staph. aureus* ranged from 64 to 256 dilution units. When the activity of the culture fluid approached 256 dilution units, the liquid was decanted with suitable precautions to prevent contamination of the cultures and each mycelial mat was reflooded with 1 l. of fresh sterile culture solution. Within 6 to 10 days the activity justified further decantation and reflooding. Several mats were reflooded as many as 20 times in the

course of a year with as rapid and as great production of antibacterial substances during the last reflood period as during the first. Maximum activity of culture liquid in reflooded flasks was reached in 2 weeks or less; extending the period to 8 weeks did not result in a material increase in activity. An extract of the mycelium prepared by grinding with sand and water exhibited considerable antibacterial activity for *Staph. aureus*.

Preliminary experiments showed that the antibacterial activity was increased 4 to 8 times by boiling the culture liquid for 10 minutes. For example, culture liquid with an activity for *Staph. aureus* of 64 dilution units per ml. was mixed with an equal volume of 0.1 M. phosphate buffers to produce pH 3.0 or pH 6.5. An equal volume of sodium bicarbonate-sodium carbonate buffer solution of pH 8.5 was added to some of the liquid and to another aliquot an equal volume of 2 per cent  $\text{Na}_2\text{CO}_3$  to reach approximately pH 11.0. Each of these solutions was allowed to stand 1 hr. at 25°C., 1 hr. at 60°C. and 10 minutes at 100°C., neutralized and assayed. Boiling at 100°C. at pH 3.0 and 6.5 increased the activity on *Staph. aureus* 4 times, did not affect it at pH 8.5 and reduced it at pH 11.0. The following table in which the activity on *Staph. aureus* is given in dilution units per ml. summarizes the effects of pH and temperature on the culture liquid in this experiment.

pH	1 HR., 25°C.	1 HR., 60°C.	10 MINUTES, 100°C.
3.0	32	64	128
6.5	64	32	256
8.5	16	32	16
11.0	16	2	< 2

There appeared to be two antibiotic substances in the boiled culture liquid of *P. biformis*; one neutral in character; the other, acid. It was found that most of the active material could be extracted by organic solvents from slightly acid, neutral or slightly alkaline solutions; that is, it behaved as a neutral substance. However, by extracting the culture liquid twice with  $\text{CHCl}_3$  at pH 6 to 7, acidifying the residual culture liquid to pH 2, or lower, and reextracting with  $\text{CHCl}_3$ , the presence of a small amount of an antibiotic substance acidic in nature was demonstrated. The neutral substance was named biformin and the acidic, biforminic acid.

It was found, further, that the antibacterial activity was completely lost if the culture liquid, or extracts from it, were dried. The  $\text{CHCl}_3$  extracts, even though dried in a high vacuum, formed a black, insoluble product and lost their activity completely.

*Concentration of Antibacterial Substances.*—The concentration procedure eventually developed and generally used in our experiments was as follows: The pooled culture liquid (pH 4.0) from 20 Fernbach flasks was boiled for 10 minutes, cooled and extracted with 0.1 volume of  $\text{CHCl}_3$  to remove biformin. The residual culture liquid was acidified to pH 2.0 or



less and reextracted with 0.1 volume of  $\text{CHCl}_3$ . This  $\text{CHCl}_3$  solution contained the biforminic acid. The aqueous extracted solution had little activity and was discarded.

The  $\text{CHCl}_3$  solution containing the biformin (neutral antibacterial substance) was evaporated at room temperature to small volume in a large crystallizing dish by blowing a stream of warm air from a hair-drier over the surface of the liquid. Before complete drying, the  $\text{CHCl}_3$  was covered by a layer of water and evaporation continued until all of the chloroform was removed. The turbid, brownish aqueous solution was filtered to remove water-insoluble substances. The filtrate was clarified and sterilized by filtration through a Seitz sterilizing pad. The resulting pale yellow solution had an activity against *Staph. aureus* of from 900 to 1750 dilution units per mg. of solids.

The biformin was further purified by precipitating with silver nitrate and recovering it from the silver precipitate. Two procedures were followed, both of which appeared satisfactory. An aqueous solution of crude biformin, prepared as described above, was acidified with  $\text{HNO}_3$  and silver nitrate was added. An alternate method was to stir a  $\text{CHCl}_3$  extract of culture fluid containing biformin with one quarter volume of 0.17 per cent  $\text{AgNO}_3$  for four hours.

The yellow silver compound was found to darken on drying and biformin could not be recovered from the brownish material. It seemed to be stable if kept under water. Biformin was recovered from the silver compound by suspending it in a large volume of water, stirring vigorously with an electric stirrer and adding KI and HCl slowly. Stirring was continued for an hour after the addition of the KI and HCl. The mixture of silver iodide, biformin and water was neutralized and extracted several times with ether. The ether was removed by rapid evaporation over a layer of water with stirring. The aqueous solution was filtered through a Seitz filter to remove finely divided solids and to sterilize the solution. A solution of biformin prepared in this way assayed 3600 dilution units per milligram against *Staph. aureus*.

The chloroform solution containing biforminic acid (the acid antibacterial substance) was extracted twice with one-tenth volume of 1 per cent sodium bicarbonate solution. The combined bicarbonate solutions were extracted twice with an equal volume of ether, which was discarded. The bicarbonate solution was then acidified to less than pH 2 and the turbid aqueous solution extracted three times with an equal volume of ether. The ether extracts were combined and washed several times with water. The biforminic acid was extracted from the ether solution by a small amount of one per cent sodium bicarbonate solution. This antibiotic substance was nearly as sensitive as biformin to drying.

The antibacterial activities of biformin and biforminic acid were similar. This was the justification for naming the acid substance biforminic acid.

Biforminic acid accounted for less than 10 per cent of the antibacterial activity of the boiled culture liquid. The biforminic acid content of the culture liquid, as far as our observations indicated, was not materially affected by boiling. The increased activity on boiling the culture liquid was the result of the formation of biformin. The precursor of biformin was not extracted by  $\text{CHCl}_3$  or methyl isobutyl ketone and was inactive on *Staph. aureus*.

*Chemical Properties of Biformin.*—Biformin was found to be soluble in water, very soluble in ether, chloroform, methyl-isobutyl-ketone and alcohol. It formed nearly colorless solutions in water. It was not volatile with steam. Its activity was unaffected by heating to boiling in 0.01 N acid or alkali. Biformin was quite stable in dilute aqueous solution, but unstable in concentrated aqueous solution. Its instability on drying and in concentrated solution and its ability to form a silver compound, all suggest that it is a highly unsaturated substance. The black, insoluble product which resulted from drying biformin, was interpreted to be a polymerization product.

Neither biformin nor biforminic acid gave a colored compound with potassium cyanide solution. They did not release iodine from cold hydriodic acid. A biforminic acid preparation of low purity gave a brown color with aqueous ferric chloride; biformin did not react.

*Antibacterial Activity of Biformin and Biforminic Acid.*—Both biformin and biforminic acid were active against a wide variety of bacteria. The activities were determined by serial dilution and are given in the following table:

ORGANISM	INHIBITORY CONCENTRATION IN $\mu\text{G}$ PER ML		
	BIFORMIN 121 3	BIFORMIN 114-417	BIFORMINIC ACID 112 71
<i>Bacillus mycoides</i>	5	13	3 4
<i>Bacillus subtilis</i>	0 04	0 04	0 2
<i>Escherichia coli</i>	1 6	0 6	3 4
<i>Klebsiella pneumoniae</i>	1 6	0 6	6 8
<i>Photobacterium fischeri</i>	0 02	..	
<i>Pseudomonas aeruginosa</i>	50	32	220
<i>Mycobacterium phlei</i>	0 6	> 2 5	0 8
<i>Mycobacterium smegmatis</i> (smegma)	3	3 5	6 8
<i>Staphylococcus aureus</i>	0 3	0 8	0 7

Results for two of the purest preparations of biformin prepared through the silver compound are given. The biforminic acid was a concentrate that had good activity. The results are reported as the minimum concentration in  $\mu\text{g}$ . per ml. that caused inhibition of growth of the test bacteria for 24 hours. The *Pseudomonas aeruginosa* was obtained through the courtesy of Merck & Co., Inc.; the *Mycobacterium phlei* was obtained from Dr. G. Rake; the *M. smegmatis* (smegma) was a strain that had been carried in culture on



an egg medium at Smith College for many years and was furnished by Dr. Elinor V. Smith.

*Pseud. aeruginosa* was the most resistant organism tested, requiring 32 to 50  $\mu$ g. per ml. for inhibition. *B. subtilis*, *E. coli*, *K. pneumoniae*, *M. phlei*, *M. smegmatis* (smegma), *Photo. fischeri* and *Staph. aureus* were inhibited by less than 5  $\mu$ g. per ml. *B. mycoides* was somewhat more resistant.

We were not successful in developing strains of *Staph. aureus* resistant to biformin.

**Antifungal Activity.**—*P. biformis* and biformin exhibited considerable antifungal activity. This was suggested by the observation that contamination of cultures of *P. biformis* after decanting culture liquid and reflooding was extremely rare. Inhibition of various fungi growing in the vicinity of colonies of *P. biformis* was observed. No growth of *Trichophyton mentagrophytes* occurred in a nutrient agar containing 4 dilution units (*Staph. aureus*) of biformin per ml.<sup>4</sup>

**Mycobacterium Tuberculosis.**—Through the courtesy of Dr. Ralph R. Mellon, Institute of Pathology, Western Pennsylvania Hospital, Pittsburgh, Pa., preliminary tests were carried out by Miss Jean Onslow on the activity of *P. biformis* on *M. tuberculosis*. Agar discs, cut from a 6-day old colony grown on Czapek-Dox agar medium supplemented with corn steep solids, inhibited an avirulent strain (H 234) of *M. tuberculosis* on a yeast-peptone medium. Discs from a 4-day old culture of the fungus were found to give zones of inhibition on virulent *M. tuberculosis* H 37 grown on Herrold egg agar. A comparison of the activity on *Staph. aureus* and *M. tuberculosis* on the two media is given in the following table. The discs were 5.5 mm. in diameter and cut on a radius extending from the center of the colony. The first two discs came from within the limits of the fungous colony. The diameter of the zones of inhibition is given in mm.

ORGANISM AND MEDIUM	DISC					
	1	2	3	4	5	6
<i>Staph. aureus</i>						
Yeast peptone	22	22	22	12	Halo	
<i>M. tuberculosis</i> (H 234)						
Yeast peptone	18	15	11	9	0	
<i>Staph. aureus</i>						
Herrold egg agar	9	9	0	0	0	0
<i>M. tuberculosis</i> (H 37)						
Herrold egg agar	16	14	14	0	0	0

Boiled culture liquid with an activity of 256 dilution units for *Staph. aureus* showed some activity in Kirchner's medium for both strains of *M. tuberculosis*.

Further experiments<sup>6</sup> with a biformin sample prepared through the silver compound indicated the activity *in vitro* to be as great or greater on *M. tuberculosis* as on *Staph. aureus*. This is shown in the following table, in which the minimum inhibitory concentrations are given in  $\mu\text{g. per ml.}$

SAMPLE NO.	COMPOUND	<i>M.</i> <i>phlei</i>	<i>M.</i> <i>smegmatis</i>	<i>M.</i> <i>tuberculosis</i> H 37 Rv	<i>Staph.</i> <i>aureus</i> (H)
121-3	Biformin	1.4	1.8	0.56	0.97

*Staph. aureus* was grown in beef heart broth and the Mycobacteria in a modified Kirchner's medium.

*Effect of Blood on Activity of Biformin.*—The addition of 5 per cent calf serum to beef heart broth had little or no effect on the activity of biformin on *Staph. aureus*. The addition of 5 per cent defibrinated rabbit blood reduced the activity to 0.1 that in the both.

*Animal Toxicity.*—Given intravenously, 10.7 mg./kg. of sample 121-3 caused no deaths in 5 mice used for each test. However, 12.5 mg./kg. of sample 121-3 killed 3 of 10, 15.0 mg./kg. killed 6 of 10 mice and 18.8 mg./kg. killed 5 of 5. Given intraperitoneally to small numbers of animals, 9.4 mg./kg. of sample 121-3 killed 4 of 5; with 12.5 mg./kg. of sample 121-3, 2 of 5 were killed and 37.5 mg./kg. killed 5 out of 5. At the lower dosage the deaths were delayed. Administered subcutaneously or intraperitoneally, 7.15 mg./kg. per day over a period of 5 days caused no deaths of 5 treated mice, while 4.68 mg./kg. per day, administered intraperitoneally to younger mice for 12 days, resulted in 1 death in 10 mice.

One of us (F. K.) suffered from severe dermatitis involving swelling and watery blisters after contact with preparations of biformin.

*Chemotherapeutic Action.*—No chemotherapeutic benefits were observed in mice of preparation 121-3 against *Staph. aureus* (Smith) or *M. tuberculosis* H 37 infections in mice. The lack of activity *in vivo* was probably related to the action of blood in reducing the activity of biformin (see above).

Preparation 121-3 was given intraperitoneally and subcutaneously at 7.5 mg./kg. in tests with *Staph. aureus* (Smith). The experiment was repeated with 3.75 mg./kg. intraperitoneally with one group of mice and 7.5 mg./kg. subcutaneously with another. In tests with *M. tuberculosis* preparation 121-3 was given intraperitoneally at 9.38 mg./kg. daily for 3 days and reduced to 4.69 mg./kg. per day until death of the animals. The experiment was repeated using an intraperitoneal injection of 2.5 to 3.75 mg./kg. daily.

*Summary.*—*Polyporus biformis* produces two antibiotic substances in culture liquids. These have been named biformin and biforminic acid. Both substances inhibit a number of gram negative, gram positive and acid-fast bacteria. The best preparations of biformin inhibited *Staphylococcus aureus* in beef broth at less than 1  $\mu\text{g. per ml.}$  and were equally active on *Mycobacterium tuberculosis*. Serum did not affect the activity *in vitro* but

whole blood reduced the activity materially. No chemotherapeutic action was observed against *Staph. aureus* or *M. tuberculosis* infections in mice.

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<sup>1</sup> Robbins, W. J., Hervey, A., Davidson, R. W., Ma, R., and Robbins, W. C., *Bull. Torrey Bot. Club*, 72, 165-190 (1945).

<sup>2</sup> This fungus was obtained from Ross W. Davidson and is numbered 71423R in his collection.

<sup>3</sup> Robbins, W. J., Kavanagh, F., and Hervey, A., these PROCEEDINGS, 33, 171-176 (1947).

<sup>4</sup> The authors are indebted to Dr. Ilda McVeigh for this experiment.

<sup>5</sup> The authors are indebted to Miss C. M. McKee, The Division of Microbiology, The Squibb Institute for Medical Research, for assistance in these experiments and those on blood, animal toxicity and therapeutic action.

## THE POLE CELLS OF DIPTERA, THEIR FATE AND SIGNIFICANCE

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Numerous investigations of the pole cells of Diptera, *Miasor*, *Chironomus*, *Calliphora*, *Lucilia* and *Drosophila* have amply verified the classical observations of Metschnikoff (1855, 1866), Leuckhart (1865) and Balbiani (1885) that these are the source of the germ cells in the gonads of flies.<sup>1</sup> The generalization of the early separation of germ and somatic lines of cells has become so firmly established among biologists that certain very fundamental facts concerning the pole cells have been overlooked in spite of a number of important pieces of information reported in recent investigations by Rabinowitz (1941), Sonnenblick (1941), and Aboim (1945).<sup>2</sup> The last named alone has recognized the possibility that some of the pole cells may have some other developmental fate than that classically demonstrated.

In the course of a detailed study of the normal embryology of *Drosophila* the author has obtained embryological and cytological evidence which establishes the pole cells in a new light and at the same time fills out our hitherto incomplete and unsatisfactory understanding of the nature and origin of the mid-gut of higher Diptera.

The formation of the pole cells in *Drosophila melanogaster* was first described by Huettnier (1923). Subsequently Rabinowitz (1941) studied them in great detail providing data to show that far more pole cells are formed than ever enter the gonads. The number at the incipient blastoderm stage is 39-73, average 55, but prior to the invaginations which are the

most conspicuous feature of gastrulation, some of the pole cells migrate between the posterior blastoderm cells to the outer surface of the yolk mass, leaving 20-47, average 39, pole cells to be carried into the proctodaeal-amniotic invagination. Of these pole cells only slightly more than half are included in the gonads according to Sonnenblick (1941) and Aboim (1945). The non-included pole cells are not easily followed in iron hematoxylin preparations, and Sonnenblick suggests they are lost in the gut contained yolk. Rabinowitz states that the early migrating pole cells become lost and degenerate in the yolk. Aboim observed no signs of degenerating pole cells in the gut, but he was not able otherwise to establish their fate.

The observations here reported elucidate the fate of these "lost" pole cells and clarify the nature of the first pole cell movements between the blastoderm cells to the interior of the egg. A detailed account illustrated with photomicrographs will appear elsewhere. This report is based on a study of a series of sections of timed embryos of the Oregon R. wild stock of *D. melanogaster* which were silver impregnated by the Bodian technique, as described by Poulson (1945).<sup>3</sup> This method provides remarkably clear differentiation of various cell and tissue types. The pole cells stand out distinctively in such preparations and there is little likelihood of confusion with other cells, both with regard to nuclear structure and appearance of cytoplasm.

After the dorsal and lateral movement (7-9 hours) of the germinal pole cells into the mesoderm at the level of the 10th segment where they become incorporated in the gonads, the remaining pole cells are found in association with the posterior mid-gut rudiment. They come to take up a position at the anterior edge of the posterior rudiment as it moves to unite with the anterior rudiment of mid-gut between the 9th and 10th hours. They form two groups (on either side and not so noticeable in iron-hematoxylin preparations, but conspicuous in Bodian treated sections) at the points of union of the mid-gut rudiments. At first the groups look like small gonads, but this appearance rapidly changes as the mid-gut cells undergo the movements and form changes which complete the enclosure of the yolk. The pole cells retain their characteristic appearance, and it can be seen that at the time at which the sac-like gut begins to change its form to a long tube one of the first constrictions is at the level of the intestinal pole cells. With the completion of the form-change in the mid-gut the pole cells are seen to form the inner epithelium of the mid-section of the mid-gut. They remain characteristically different in size and form from the cells of the more anterior and posterior sections of the mid-gut and clearly correspond to the large cells of that region of the larval mid-gut designated by Marie Strasburger (1932) as the "Mitte."<sup>4</sup> Strasburger's observations on the physiology of this section of the gut set it off from the other parts of the mid-gut as the region of acid secretion. The physiological studies of Hobson (1931) on the



same region of the gut of *Lucilia* characterize it as a region of low pH (3.2) and of little or no proteolytic enzyme activity.<sup>5</sup> Studies of Waterhouse (1945) on the differential uptake of metallic ions by certain cells in this region of the mid-gut of *Lucilia cuprina* are of interest in view of its origin.<sup>6</sup> The fate of this region in metamorphosis to the adult requires careful study, although from Robertson's (1936) account of metamorphosis of the mid-gut<sup>7</sup> it seems likely that these cells are lost with most of the other larval mid-gut epithelial cells.

The early entry of pole cells between the posterior blastoderm cells to the interior is interpreted as the first of the series of movements in gastrulation in the eggs of *Drosophila* and other higher Diptera. These cells remain at the posterior surface of the yolk spreading out on it and later moving dorso-anteriorly with the extending embryo. They become incorporated also with the posterior rudiment of mid-gut and thus a part of the definitive larval intestine. They come to lie along with the "lost" pole cells in the middle region of the mid-gut and are difficult to distinguish from them.

Experimental proof of this should be obtainable in embryos and larvae in which the pole cells have been removed or killed by cautery or ultra-violet light; in these the middle section of the mid-gut must be reduced or missing. Such material is already in the hands of Geigy (1931) and Aboim (1945) who had followed the development of agametic gonads of *Drosophila* following ultra-violet treatment of pole cells.<sup>8</sup> It is hoped that this experimental evidence will soon be forthcoming.

It is no longer possible to interpret the posterior polar plasm, or oosome, and its included granules as germ cell determinants in the original sense of Hegner and his predecessors, or even in the modified sense of Huettnner (1923). The pole cells must be regarded in a new light as potential, but not as completely determined, germ cells. The latter is true only when, as Aboim (1945) has so beautifully demonstrated, they enter the lateral mesoderm. When they do not, they become a specialized section of the mid-gut whose function may well repay careful investigation. Although no cytological differences have been demonstrated between those cells which are observed to leave the mid-gut and those which remain there, physiological and biochemical differences may well be sought at an early stage.

<sup>1</sup> For references and discussion of early literature on the pole cells see: Huettnner, A. F., *Jour. Morph.*, **37**, 385-423 (1923); Wilson, E. B., *The Cell in Development and Heredity*, 3rd ed., Macmillan Co., New York, 1232 pp. (1928).

<sup>2</sup> Rabinowitz, M., *Jour. Morph.*, **69**, 1-49 (1941); Sonnenblick, B. P., these PROCEEDINGS **27**, 484-489 (1941); Aboim, A. N., *Revue Suisse de Zool.*, **52**, 53-154 (1945).

<sup>3</sup> Poulson, D. F., *Trans. Conn. Acad. Arts. Sci.*, **36**, 449-487 (1945).

<sup>4</sup> Strasburger, Marie, *Z. f. wiss. Zool.*, **140**, 539-649 (1932).

<sup>5</sup> Hobson, R. P., *Jour. Exp. Biol.*, **8**, 109-123 (1931).

<sup>6</sup> Waterhouse, D. F., *Conn. Sci. Ind. Res. (Australia)*, Bull. **191**, 1-20 (1945).

<sup>7</sup> Robertson, C. W., *Jour. Morph.*, **59**, 351-399 (1936).

<sup>8</sup> Geigy, R., *Revue Suisse de Zool.*, **38**, 187-288 (1931); Aboim, A. N., *loc. cit.*

*A GENERAL CLASS OF PROBLEMS IN CONFORMAL MAPPING*

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A function  $f(z)$  which is defined in the unit circle is said to be schlicht there if the relations  $f(z_1) = f(z_2)$ ,  $|z_1| < 1$ ,  $|z_2| < 1$  imply that  $z_1 = z_2$ . We shall say that a function

$$f(z) = \sum_{\nu=1}^{\infty} a_{\nu} z^{\nu}, \quad |z| < 1, \quad (1)$$

belongs to class  $S$  if  $f(z)$  is regular and schlicht in  $|z| < 1$  and  $a_1 = 1$ .

Let  $\mathbf{R}$  be a closed set lying in  $|z| < 1$ , and let  $\Psi_{\nu}(\tau)$  be a measure function defined in the space  $\mathbf{R}$ . Given an integer  $n$  there is a number  $M = M_n$  such that  $|f^{(\nu)}(z)| \leq M$  for all  $z \in \mathbf{R}$  and for  $\nu = 0, 1, 2, \dots, n$  when  $f(z)$  belongs to class  $S$ . Let  $F_{\nu}(\zeta_0, \bar{\zeta}_0, \dots, \zeta_n, \bar{\zeta}_n)$  denote a complex-valued function which is continuous together with its first-order partial derivatives in an open set containing the closed set  $|\zeta_{\nu}| \leq M$ , ( $\nu = 0, 1, \dots, n$ ).

Given the functions  $F_1, F_2, \dots, F_m$  and the measure functions  $\Psi_1, \Psi_2, \dots, \Psi_m$ , let

$$P_{\nu} = \int_{\mathbf{R}} F_{\nu}(f(z), f'(z), \dots, f^{(n)}(z), \bar{f}^{(n)}(z)) d\Psi_{\nu}, \quad (2)$$

for  $\nu = 1, 2, 3, \dots, m$ . If  $f(z)$  belongs to class  $S$  then the point

$$P_f = (P_1, P_2, \dots, P_m)$$

is a point in a Euclidean space of  $2m$  real dimensions, and the point  $P_f$  is said to belong to  $f(z)$ . As  $f(z)$  ranges over  $S$ , the point  $P_f$  belonging to  $f(z)$  ranges over a set which we call  $P$ . Since the class  $S$  is compact, the set  $P$  is closed and bounded. The problem is to find the region  $P$ .

Many of the problems on schlicht functions which concern the values taken in the interior of the unit circle, as opposed to boundary-value problems, are subsumed under this general formulation. Since special problems included in this general statement can often be carried further toward complete solution, this note will discuss several specific problems of this type and it will be observed that the method applied to these problems can be extended, at least in part, to the general problem. The region of variability of the coefficients  $(a_2, a_3, \dots, a_n)$  of functions belonging to class  $S$  is one problem of this type, and it has been discussed in a previous note by the authors.<sup>1</sup>

If  $z_1$  is a point of  $|z| < 1$ , an important problem is to find the region of variability of  $f'(z_1)$  when  $f(z)$  belongs to class  $S$ . Let  $f'(z_1) = p + iq$ , where  $p$  and  $q$  are real. Then for fixed  $z_1$ , the point  $(p, q)$  ranges over a



closed plane set  $P$  when  $f$  ranges over  $S$ . Let  $G(p, q)$  be continuous together with its first order partial derivatives in an open set containing  $P$ , and let the gradient of  $G$  be non-vanishing in  $P$ . Let the maximum of  $G(p, q)$  in  $P$  occur for a particular function  $f(z) \in S$ .

In a paper to appear shortly, the authors show that if  $(\alpha, \beta)$  is an analytic Jordan arc in  $|z| < 1$  not passing through  $z = 0$  or  $z = z_1$ , then for every sufficiently small complex number  $\epsilon$  there is a function  $f_\epsilon(z)$  which tends to  $f(z)$  as  $\epsilon$  tends to zero, and such that

$$f_\epsilon(z) = f(z) + \frac{\epsilon}{2\pi i} \int_\alpha^\beta \frac{p(u)}{2u^2} \left\{ \left( \frac{uf'(u)}{f(u)} \right)^2 \frac{2f^2(z)}{f(u) - f(z)} - zf'(z) \frac{u+z}{u-z} + f(z) \right\} du + \frac{\bar{\epsilon}}{2\pi i} \int_\alpha^\beta \frac{\bar{p}(u)}{2\bar{u}^2} \left\{ zf'(z) \frac{1+\bar{u}z}{1-\bar{u}z} - f(z) \right\} d\bar{u} + o(\epsilon). \quad (3)$$

Here  $p(u)$  is an arbitrary function which is analytic in a domain containing  $(\alpha, \beta)$  and vanishes at  $\alpha$  and  $\beta$ .

If  $\Delta p + i\Delta q$  is the variation of  $f'(z_1)$ , then the variation of  $G$  when  $f(z)$  is replaced by  $f_\epsilon(z)$  is given by

$$\Delta G = \frac{\partial G}{\partial p} \Delta p + \frac{\partial G}{\partial q} \Delta q + o(\epsilon)$$

or

$$\Delta G = \operatorname{Re}\{G_1 \Delta(f'(z_1))\} + o(\epsilon), \quad (4)$$

where

$$G_1 = \frac{\partial G}{\partial p} - i \frac{\partial G}{\partial q}.$$

The variation  $\Delta(f'(z_1))$  of  $f'(z_1)$  can be obtained by differentiating (3) with respect to  $z$  and setting  $z = z_1$ . If we substitute this in relation (4) we find that  $\Delta G$  is equal to the real part of an expression of the form

$$\frac{\epsilon}{2\pi i} \int_\alpha^\beta A(z_1, u) du + \frac{\bar{\epsilon}}{2\pi i} \int_\alpha^\beta B(z_1, \bar{u}) d\bar{u} + o(\epsilon).$$

We may replace the second term by its conjugate without changing the real part, so  $\Delta G$  is, within an error  $o(\epsilon)$ , equal to the real part of

$$\frac{\epsilon}{2\pi i} \int_\alpha^\beta \frac{p(u)}{2u^2} \left\{ \left( \frac{uf'(u)}{f(u)} \right)^2 P \frac{2f(u) - f(z_1)}{[f(u) - f(z_1)]^2} - Q \frac{u + z_1}{u - z_1} - \bar{Q} \frac{1 + u\bar{z}_1}{1 - u\bar{z}_1} - M \frac{2u - z_1}{(u - z_1)^2} - \bar{M} \frac{u(2 - u\bar{z}_1)}{(1 - u\bar{z}_1)^2} \right\} du, \quad (5)$$

Here  $P = 2G_1 f'(z_1) f(z_1)$ ,  $Q = G_1 z_1 f''(z_1)$  and  $M = 2G_1 z_1 f'(z_1)$ . Since  $G$  attains its maximum for the function  $f(z)$ , the real part of (5) is non-

negative for all small  $\epsilon$  so (5) must be equal to zero. Since  $p(u)$  is an arbitrary analytic function subject to the conditions stated, we see that  $f(z)$  must satisfy the differential equation

$$P \left( \frac{zf'(z)}{f(z)} \right)^2 \frac{2f(z) - f(z_1)}{[f(z) - f'(z_1)]^2} = Q \frac{z + z_1}{z - z_1} + \bar{Q} \frac{1 + z\bar{z}_1}{1 - z\bar{z}_1} + M \frac{2z - z_1}{(z - z_1)^2} + \bar{M} \frac{z(2 - z\bar{z}_1)}{(1 - z\bar{z}_1)^2} \quad (6)$$

where  $z$  has been written in place of  $u$ .

The right-hand side of (6) is real on the unit circle  $|z| = 1$ , and can be shown to be non-negative there with at least one zero of even order on  $|z| = 1$ . If we set  $w = f(z)$ , then this differential equation can be written in the form

$$A \left( \frac{dw}{dz} \right)^2 \frac{2w - \alpha}{w^2(w - \alpha)^2} = \frac{(z - e^{i\psi})^2(z - z_2)(z\bar{z}_2 - 1)}{(z - z_1)^2(1 - z\bar{z}_1)^2}. \quad (7)$$

Here  $A$ ,  $\alpha$ ,  $\psi$ ,  $z_2$  are constants with  $\psi$  real,  $|z_2| \leq 1$ , and  $\alpha = w(z_1)$ . The conditions that  $w(z)$  is normalized at the origin and that  $(w - \alpha)/(z - z_1)$  approaches  $w'(z_1)$  as  $z$  approaches  $z_1$  imply that

$$A = -\alpha e^{2i\psi} z_2 z_1^{-2}$$

and

$$A = \alpha(z_1 - z_2)(z_1\bar{z}_2 - 1)(z_1 - e^{i\psi})^2 z_1^{-2}(1 - |z_1|^2)^{-2}.$$

The point  $z_1$  can be taken real and positive without loss of generality.

The variables in equation (7) may be separated and the integrals expressed in terms of elementary functions. Thus the region of variability of  $f'(z_1)$  is obtained exactly at all points of the unit circle  $|z| < 1$ . A function belonging to a boundary point of this region maps  $|z| < 1$  onto the  $w$ -plane minus analytic slits which have at most a finite number of critical points. The authors hope to discuss this region in greater detail in a later paper.

If  $f(z)$  belongs to class  $S$  the area of the map by  $w = f(z)$  of the circle  $|z| \leq a$ ,  $0 < a < 1$ , is given by the expression

$$A = \int_0^a \int_{-\pi}^{\pi} |f'(re^{i\theta})|^2 r dr d\theta = \frac{a}{4} \frac{d}{da} \int_{-\pi}^{\pi} |f(ae^{i\theta})|^2 d\theta.$$

To illustrate the principle with equations which are not too complicated, consider the allied problem of maximizing the integral

$$B = \int_{-\pi}^{\pi} |f(ae^{i\theta})|^2 d\theta \quad (8)$$

when  $f(z)$  ranges over  $S$ . Let the maximum of  $B$  occur for a particular function  $f(z) \in S$ . If the variation (3) is written in the form

$$f_\epsilon(z) = f(z) + \epsilon\Phi(z) + \bar{\epsilon}\Psi(z) + o(\epsilon)$$

then, substituting  $f_\epsilon(z)$  for  $f(z)$  in (8), one obtains

$$B_\epsilon = B + 2\operatorname{Re}\left\{\epsilon \int_{-\pi}^{\pi} (f\Phi + f\bar{\Psi})d\theta\right\} + o(\epsilon).$$

It therefore follows that

$$\int_{-\pi}^{\pi} (f\Phi + f\bar{\Psi})d\theta = 0$$

and if the function  $p(u)$  is "peaked," it follows after some manipulation that

$$\begin{aligned} \left(\frac{zf'(z)}{f(z)}\right)^2 \int_{-\pi}^{\pi} \frac{2f(ae^{i\theta})|f(ae^{i\theta})|^2}{f(z) - f(ae^{i\theta})} d\theta = \int_{-\pi}^{\pi} \left\{ -2|f(ae^{i\theta})|^2 + \right. \\ \left. ae^{i\theta}f'(ae^{i\theta})\bar{f}(ae^{i\theta}) \frac{z + ae^{i\theta}}{z - ae^{i\theta}} + \right. \\ \left. ae^{-i\theta}\bar{f}'(ae^{i\theta})f(ae^{i\theta}) \frac{1 + zae^{-i\theta}}{1 - zae^{-i\theta}} \right\} d\theta. \end{aligned}$$

Here the independent variable  $z$  has been written in place of  $u$ .

Several sub-classes of the class  $S$  are often studied, of which we mention three:

$S_M$ , the class of functions  $f(z) \in S$  which satisfy  $|f(z)| \leq M$  in  $|z| < 1$ , where  $M$  is some constant exceeding 1.

$S_r$ , the class of functions  $f(z) \in S$  which are real for real  $z$ .

$S_0$ , the class of functions  $f(z) \in S$  which satisfy  $f(-z) = -f(z)$ .

For functions belonging to  $S_M$  we have in place of (3)

$$\begin{aligned} t_\epsilon(z) = f(z) + \frac{\bar{\epsilon}}{2\pi i} \int_a^b \frac{p(u)}{2u^2} \left\{ \left( \frac{uf'(u)}{f(u)} \right)^2 \frac{2f^2(z)}{f(u) - f(z)} - zf'(z) \frac{u + z}{u - z} + \right. \\ \left. f(z) \right\} du - \frac{\bar{\epsilon}}{2\pi i} \int_a^b \frac{\bar{p}(u)}{2\bar{u}^2} \left\{ \left( \frac{\bar{u}\bar{f}'(u)}{\bar{f}(u)} \right)^2 \frac{2\bar{f}(u)\bar{f}(z)}{M^2 - \bar{f}(u)\bar{f}(z)} - \right. \\ \left. zf'(z) \frac{1 + \bar{u}z}{1 - \bar{u}z} + f(z) \right\} d\bar{u} + o(\epsilon), \quad (9) \end{aligned}$$

where, however,

$$\operatorname{Re} \left\{ \frac{\epsilon}{2\pi i} \int_a^b \frac{p(u)}{2u^2} \left[ 1 - \left( \frac{uf'(u)}{f(u)} \right)^2 \right] du \right\} + o(\epsilon) \leq 0. \quad (10)$$

Condition (10) restricts the small complex number  $\epsilon$  to lie in a half-plane. Using (9) and (10), the differential equation satisfied by a function  $(z)f$

of class  $S_M$  which belongs to a boundary point of the region of variability of  $f'(z_1)$ ,  $f \in S_M$ , is of the form

$$\left(\frac{zf'(z)}{f(z)}\right)^2 \left\{ \lambda \frac{2f(z) - \alpha}{(f(z) - \alpha)^2} + \bar{\lambda} \frac{f(z)(2M^2 - \bar{\alpha}f(z))}{(M^2 - \bar{\alpha}f(z))^2} + \rho \right\} = \\ \beta \frac{2z - z_1}{(z - z_1)^2} + \bar{\beta} \frac{2z - z^2\bar{z}_1}{(1 - z\bar{z}_1)^2} + \gamma \frac{z + z_1}{z - z_1} + \bar{\gamma} \frac{1 + z\bar{z}_1}{1 - z\bar{z}_1} + \rho$$

where  $\alpha = f(z_1)$ ,  $\lambda$ ,  $\beta$  and  $\gamma$  are complex constants and  $\rho$  is a non-negative constant. The variation can also be modified such that, if  $f(z)$  belongs to  $S_r$  or to  $S_0$ , so does  $f_*(z)$ . Corresponding differential equations are then obtained for functions belonging to these classes.

The general problem stated in the beginning of this note leads to a differential equation for extremal schlicht functions which is of the form

$$\left(\frac{dw}{dz}\right)^2 N(w) = Q(z).$$

Here  $N(w)$  and  $Q(z)$  will be rational functions whenever (2) reduces to the form

$$P_\nu = \sum_{j=1}^k F_\nu(f(z_j), f'(z_j), \dots, f^{(n)}(z_j), f^{(n)}(z_j)) \quad (13)$$

for  $\nu = 1, 2, \dots, m$ . This is the most important case at the present time, and problems concerning the region of variability  $P$  of the point  $(P_1, P_2, \dots, P_m)$ , where  $P_\nu$  is given by (13), can be further classified according to the order  $n$  of the highest derivative involved.

\* This note was written during the time that the authors were under contract with the Office of Naval Research.

<sup>1</sup> *The coefficients of schlicht functions III*, these PROCEEDINGS, 32, 111-116 (1946).

## CONTINUOUS PARTICLES

BY M. HESSABY

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1. *The Gravitational Field of an Uncharged Mass.*—In the theory of the electromagnetic field an antisymmetric tensor  $F_{\mu\nu}$  is defined by the relations

$$F_{\mu\nu} = \frac{\partial\phi_\mu}{\partial x_\nu} - \frac{\partial\phi_\nu}{\partial x_\mu} \quad (1)$$

where  $\phi_\mu$  is the vector potential. The charge and current vector is

$$J^\mu = F^{\mu\nu}{}_{,\nu} \quad (2)$$

and the energy tensor is

$$U_\mu{}^\nu = -F^\mu{}^\alpha F_{\nu\alpha} + 1/4 g_\mu{}^\nu F^{\alpha\beta} F_{\alpha\beta} \quad (3)$$

which gives

$$U_\mu{}^\nu{}_{,\nu} = F_{\mu\alpha} F^{\alpha\nu}{}_{,\nu} = F_{\mu\alpha} J^\alpha \quad (4)$$

and also by contraction yields

$$U = 0. \quad (5)$$

We postulate the validity of the expression (3) for the gravitational energy tensor, subject to the supplementary condition

$$U_\mu{}^\nu{}_{,\nu} = 0 \quad (6)$$

corresponding to the fact that there is no electric charge. We postulate further that the existence of an electromagnetic field corresponds to the condition

$$U_\mu{}^\nu{}_{,\nu} \neq 0 \text{ at that point.} \quad (7)$$

Identifying therefore  $U_\mu{}^\nu$  with the gravitational energy tensor  $(-R_\mu{}^\nu + 1/2 g_\mu{}^\nu R)$ , we see that we must have  $R = 0$ , and hence  $-R_\mu{}^\nu = U_\mu{}^\nu$ , which shows that now  $R_\mu{}^\nu{}_{,\nu} = 0$ , in agreement with the Bianchi identities. The form (1) suggests the possibility of a torsional stress in addition to the radial stress. Denoting by  $P$  and  $\tau$  the radial and torsional stresses, respectively, we have the following scheme for  $F_{\mu\nu}$ :

$$F_{\mu\nu} = \begin{array}{c} \begin{array}{c} \mu \rightarrow \nu \\ \downarrow \end{array} \begin{array}{|c|c|c|c|} \hline 0 & \tau_3 & -\tau_2 & P_1 \\ \hline -\tau_3 & 0 & \tau_1 & P_2 \\ \hline \tau_2 & -\tau_1 & 0 & P_3 \\ \hline -P_1 & -P_2 & -P_3 & 0 \\ \hline \end{array} \end{array} \quad (8)$$

Consider the case where there is no torsional stress. We can write the interval around a particle with spherical symmetry in the form:

$$ds^2 = -e^\lambda dr^2 - r^2 d\theta^2 - r^2 \sin^2 \theta d\varphi^2 + e^\nu dt^2. \quad (9)$$

The only components of  $F_{\mu\nu}$ , being  $F_{14} = -F_{41} = P_1$ , we get from (3),

$$-R_1{}^1 = R_2{}^2 = R_3{}^3 = -R_4{}^4 = 1/2 P_1^2 e^{-\lambda-\nu}. \quad (10)$$

The equality  $R_1{}^1 = R_4{}^4$  leads to the relation  $\lambda' = -\nu'$ ; substituting this

result in the differential equation  $R = 0$ , we obtain, replacing  $e'$  by  $\gamma$ , the expression<sup>1</sup>

$$\gamma = 1 - \frac{2m}{r} + \frac{A}{r^2},$$

where  $m$  is the mass of the particle and  $A$  is a constant to be determined. We shall determine  $A$  by the condition that the integral of the energy density over the whole of space shall be equal to  $m$ , with a numerical factor depending on the units chosen. We have  $R^{44} = -\frac{A}{r^4\gamma}$  and

$$-\int R^{44} \sqrt{-g} dV = 4\pi A \int_0^\infty \frac{dr}{r^2 - 2mr + A} = 4\pi A \frac{1}{\sqrt{A - m^2}} \left( \frac{\pi}{2} + \arctan \sqrt{\frac{m}{A - m^2}} \right). \quad (11)$$

Setting  $A = 2m^2$ , this expression becomes equal to  $6\pi^2 m$ , and we obtain thus for  $\gamma$ ,

$$\gamma = 1 - \frac{2m}{r} + \frac{2m^2}{r^2}. \quad (12)$$

The particle appears, therefore, as a continuous distribution of energy through space, the energy density being finite at every point, and tending to zero at infinity, the greater part of the mass being concentrated near the origin. We remark that for  $r < 2m$  the field, given by  $1/2\gamma'$ , becomes repulsive. For  $r < m$ , there is a compression, and for  $r > m$ , there is a dilatation. The numerical value of  $r$  for which  $\gamma = 1$ , is, for a neutron equal to

$$1.23 \times 10^{-52} \text{ cm.} \quad (13)$$

The expression for  $\phi_4$ , is determined by the condition  $F^{4\nu}_{, \nu} = 0$  which is equivalent to

$$\frac{\partial}{\partial r} \left( \sqrt{-g} \frac{\partial \phi_4}{\partial r} \right) = 0$$

or

$$\frac{\partial}{\partial r} \left( r^2 \frac{\partial \phi_4}{\partial r} \right) = 0,$$

which gives

$$\phi_4 = \frac{2m}{r}. \quad (14)$$

Calculating the value of the invariant  $R_{\mu\nu}R^{\mu\nu}$  we find that it is equal to  $(2m/r^2)^2$ , which shows that  $\phi = 2m/r$  is an invariant function of position.



If we take into account all the components of the vector potential  $\phi_\nu$ , we are led to consider the identification of the torsional stress with the spin. The value of  $-R_4^4$  being  $\frac{2m^2}{r^4}$ , if the energy of the torsional stress can also be put in the form  $\frac{s}{r^4}$ , then the integral of the energy  $\int \frac{s}{r^4} \sqrt{-g} dV$  would take the form  $\frac{s}{m}$ , so that the torsional energy would be inversely proportional to the mass of the particle, as is the case for the spin.

2. *The Electron.*—If we consider the mass of the electron to be wholly due to its electric energy, we must determine  $F^{\mu\nu}$  by the two conditions

$$\int J^4 \sqrt{-g} dV = e \quad (15)$$

and

$$\int \frac{1}{2} E^2 \sqrt{-g} dV = m, \quad (16)$$

where  $E$  is the electrostatic field of the electron, and  $J^4$  the charge density. We have  $E = F_{14} = -F_{41}$ ; also  $J^4 = F^{4\nu}{}_{,\nu}$ , and the  $g_{\mu\nu}$  are Galilean. The two conditions are satisfied by the potential

$$\phi_4 = \frac{1}{2k} \log \eta = \frac{1}{2k} \log \left( 1 - \frac{2ke}{r} + \frac{2k^2 e^2}{r^2} \right), \quad (17)$$

where  $k$  is a constant equal to  $3.8 \times 10^{-4}$ . The field is given by  $\frac{1}{2k} \frac{\eta'}{\eta}$  and reduces to the Coulomb field at large distances. The charge and the energy densities are seen to be finite at every point, and tend to zero at infinity. The electron appears thus to be a continuous distribution of charge through space.

We remark that for a positive charge the field changes sign at a distance  $r = 3.7 \times 10^{-13}$  cm., and that two positive charges should therefore attract each other in the range  $3.7 \times 10^{-13}$  cm. to  $1.8 \times 10^{-13}$  cm.

The electrostatic stress at a point may be considered as a transverse stress on a narrow tube situated on the radius vector.

Moreover if the radial stress of an uncharged mass has a transverse Poisson effect, then a neutron might be expected to have an attraction for a positive charge.

3. *Waves in Particles.*—The wave equation of Maxwell's theory is

$$g^{\alpha\beta}(\phi_\mu)_{\alpha\beta} = J_\mu - R_\mu{}^\alpha \phi_\alpha. \quad (18)$$

For an uncharged particle  $J_\mu = 0$ . Moreover, in regions of weak fields  $R_\mu{}^\alpha$  may be neglected, and the wave equation reduces to

$$\square \phi_\mu = 0 \quad (19)$$

A solution of this equation is

$$\phi_\mu = \int_0^\infty dk \sum_{l=0}^\infty a_{klm} P_l^m \cos \theta e^{im\varphi} r^{-1/2} [\alpha J_{l+1/2}(kr) + \beta J_{l-1/2}(kr)] e^{ikct} \quad (20)$$

Imagine an observer  $O_1$  whose position coincides at time  $t$  with the center  $O$  of a particle in relative motion with respect to the observer. The form of  $\phi$  will not depend on the angle  $\varphi$ , but it may depend on the angle  $\theta$ . Let us assume that it does not depend on  $\theta$ . In that case we have  $l = 0$ , and the solution becomes:

$$\phi = \int_0^\infty a_k r^{-1/2} [\alpha J_{1/2}(kr) + \beta J_{-1/2}(kr)] e^{ikct} dk. \quad (21)$$

If we want to express this in exponential form we set  $\alpha = i$ ,  $\beta = -1$  so that

$$\phi = \int_0^\infty a_k \frac{e^{ik(r+ct)}}{kr} dk \quad (22)$$

Let us consider first (a) as a constant not depending on  $k$ . The solution will then give us again the potential  $\frac{1}{r}$ , already obtained for the static case.

We must therefore assume that  $a_k$  will be found by the observer  $O_1$  to be a function of  $k$ . We assume for  $a_k$  the form

$$a_k = \frac{1}{1 - \frac{k}{k_1}} \quad (23)$$

where  $k_1$  depends on the momentum of the particle and the nature of the medium. We obtain thus

$$\phi = \frac{A}{r} \int_0^\infty \frac{e^{ik(r+ct)}}{k - k_1} dk. \quad (24)$$

A contour integration<sup>2</sup> around the point  $k_1$  gives

$$\phi = \frac{A}{r} \left[ -2\pi \sin k_1(r+ct) + \int_0^\infty \frac{\cos k(r+ct)}{k + k_1} dk \right] \quad (25)$$

the last integral diminishes very rapidly at distances of the order of  $\frac{\pi}{k_1}$  so that at a distance of half a wave-length from the center of a moving particle a decreasing harmonic wave form is fully established and we have simply

$$\phi = -\frac{2\pi A}{r} \sin k_1(r+ct). \quad (26)$$

The particle thus appears to be surrounded by a spherical wave pattern which escorts it in its motion. The common velocity of the infinite number of waves which constitute the wave pattern, including the principal wave  $k_1$ , is  $c$ .

Substituting the form (26) in the wave equation  $\square\phi = 0$  we obtain, if  $k_1$  does not depend on time, the equation  $\Delta\phi + k_1^2\phi = 0$ . If now we set  $\lambda_1 = \frac{h}{mv}$ , in accordance with the results of experiment this expression becomes  $\Delta\phi + \frac{4\pi^2m^2v^2}{h^2}\phi = 0$ , or, replacing  $\frac{1}{2}mv^2$  by  $E - U$  in the case of small velocities,

$$\Delta\phi + \frac{8\pi^2m}{h^2}(E - U)\phi = 0 \quad (27)$$

where  $E$  is the total and  $U$  the potential energy of the particle.

*Summary.*—A reinterpretation of Maxwell's equations in general relativity leads to the deduction of a metric tensor for the field of an uncharged particle which yields a finite density of energy at every point in space, the integral of the energy over the whole of space being equal to the mass of the particle. The particle thus appears to be a continuous distribution of energy through space.

An expression is deduced for the electrostatic energy of an electron giving charge and energy densities which are finite at every point in space, the integrals over the whole of space being, respectively, equal to the charge and to the mass of the electron. An indication is found of the possibility of an attraction between two positive charges in a certain range of distance, and of the possibility of an attraction between a positive charge and an uncharged mass.

The consideration of a certain solution of the wave equation of Maxwell's theory leads to the notion of a harmonic spherical wave surrounding a particle in motion. This leads to a new interpretation of Schrödinger's wave equation.

<sup>1</sup> This form was obtained by Nordstrom and by Jeffery, who interpreted the constant  $A$  as an electric charge; see Eddington, *Math. Th. Rel.*, p. 185.

<sup>2</sup> Lamb, *Hydrodynamics*, p. 401.

## RADIO-WAVE PROPAGATION AND ELECTROMAGNETIC SURFACE WAVES

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1. *A Discrepancy and Its Resolution.*—The problem of the propagation of radio-waves along the surface of the (plane) earth was first treated in a celebrated paper by A. Sommerfeld (1909).<sup>1</sup> Let the  $(r, \varphi)$ -plane of a cylindric system of coördinates coincide with the plane of the earth surface and let the  $z$ -axis point vertically upward. Sommerfeld considered a vertically oscillating dipole (radio-antenna) in the air, close to the origin ( $z = 0, r = 0$ ) and asked what secondary waves were produced by the discontinuity due to the presence of the partially conducting earth. His investigation led to the result that the Hertzian vector  $\Pi$  describing the electromagnetic field can be divided, in either medium (air and earth), into two parts:  $\Pi = Q + P$ , where the part  $Q$  has the character of *space-waves*, which at large distances from the origin are proportional to  $R^{-1}$ , if  $R = (r^2 + z^2)^{1/2}$ . On the other hand,  $P$  is a *surface-wave*: at large distances it becomes proportional to  $r^{-1/2}$  and is restricted to the vicinity of the earth surface.

Ten years later (1919) the problem was re examined by H. Weyl<sup>2</sup> who used a somewhat different mathematical approach. He obtained a solution which was identical with Sommerfeld's space waves  $Q$  but which did not contain the surface wave  $P$ .

The reason for this discrepancy has never been satisfactorily explained. Since Weyl's method seemed mathematically simpler his result was favored by public opinion in numerous papers by other authors. Finally, in 1935, Sommerfeld himself conceded that the surface wave has no reality.<sup>3</sup> Referring to F. Noether,<sup>4</sup> he attributed this to an inaccuracy in the evaluation of his general solution. This evaluation consisted in carrying out a contour integration in which a pole of the integrand yielded the surface-wave  $P$  and two branch-cuts accounted for the space-wave  $Q$ . According to Noether's explanation the pole is so close to one of the branch-cuts that the method of integration used by Sommerfeld, possibly, was not sufficiently reliable.

The question has not only historical interest but retains even now some actuality because Sommerfeld's method was recently applied by C. Y. Fu<sup>5</sup> to the analysis of the propagation of seismic waves. In this case, the singularities, instead of nearly coalescing, as in Sommerfeld's problem, are rather far apart so that the evaluation does not present any difficulties. If Noether's suggestion is the complete explanation for the absence of

surface waves in the electrodynamic disturbances, it should not apply to seismic waves. In other words, an oscillating elastic dipole within the earth would produce, among other things, seismic surface waves. It is, therefore, important to decide what the true nature of the discrepancy is and whether the above explanation is exhaustive.

We shall show in the next section that Noether's explanation is both insufficient and unnecessary. The difficulties already arise (and can be resolved) when only the general solution is considered, and before any evaluation is attempted. It seems that the resolution of the discrepancy has been delayed so long because of the mental attitude of all involved which led them to take it for granted that one of the conflicting solutions must contain a mathematical error. This is not so: from the mathematical point of view, both Sommerfeld's and Weyl's solutions are unimpeachable. However, they represent two different physical phenomena. On the one hand, Weyl's solution ( $Q$ ) corresponds just to the wave of the oscillating dipole with its secondary space waves due to reflexion and transmission. On the other hand, Sommerfeld's solution ( $Q + P$ ) is the superposition of two independent physical systems as follows: (1) the oscillating dipole with its secondaries ( $Q$ ), (2) an electrodynamic surface wave ( $P$ ). These two systems stand in no causal relation to each other, their yoking together in one mathematical expression is purely accidental. The fact that the space-waves  $Q$ , as evaluated by Sommerfeld, are identical with those found by Weyl does not bear out Noether's suggestion that the evaluation is at fault. On the contrary, Sommerfeld's evaluation seems to be entirely adequate.

2. *Mathematical Proof.*—Sommerfeld starts from the representation of the  $z$ -component of the Hertzian function for an oscillating dipole,  $\Pi_0 = \exp. (ikR)/R$  in the form of the integral

$$\Pi_0 = \frac{1}{2} \int H(\lambda r) \exp. (\mp \sigma z) \sigma^{-1} \lambda d\lambda, \quad (1)$$

$$\sigma = (\lambda^2 - k^2)^{1/2}, \quad \sigma' = (\lambda^2 - k'^2)^{1/2}.$$

We designate here by  $k$  and  $k'$  the wave-numbers of the upper and lower medium (air and earth), while  $H$  denotes Hankel's cylindric function of the first kind and of order zero. The upper sign of the exponent refers to the case  $z > 0$ , the lower to  $z < 0$ , while the signs of the roots must be chosen so as to make the real parts of  $\sigma$  and  $\sigma'$  positive. The path of integration ( $L$ ) in the  $\lambda$ -plane is the real axis from  $-\infty$  to  $+\infty$ .

The Hertzian function determines the field components in the following way

$$E = \Pi k^2 + \nabla(\nabla \cdot \Pi), \quad H = -ik^2 c \omega^{-1} \nabla \times \Pi, \quad (2)$$

where  $c$  is the velocity of light,  $\omega$  the frequency, and the time factor  $\exp.$

$(-i\omega t)$  is omitted. This leads to the border conditions at the surface of the earth ( $z = 0$ ),

$$k^2\Pi - k'^2\Pi' = 0, \quad \partial(\Pi - \Pi')/\partial z = 0, \quad (3)$$

where  $\Pi$  is the total Hertzian function in the upper medium,  $\Pi'$  in the lower.

To satisfy the border conditions, Sommerfeld assumes the existence of a *reflected* Hertzian function in the upper medium,  $\Pi_R = \Pi - \Pi_0$ , and of a *transmitted* one  $\Pi'$ , in the lower, which differ from the expression (1) mainly by the respective factors  $f(\lambda)$  and  $f'(\lambda)$  in the integrands. These expressions satisfy the wave equations  $\nabla^2\Pi + k^2\Pi = 0$  and  $\nabla^2\Pi' + k'^2\Pi' = 0$ , while the factors can be chosen so as to fulfill the border conditions. Thus the total Hertzians in the two media become

$$\Pi = \frac{1}{2} \int H(\lambda r) \exp. (-\sigma z) \sigma^{-1} [1 + f(\lambda)] \lambda d\lambda, \quad (4)$$

$$\Pi' = \frac{1}{2} \int H(\lambda r) \exp. (\sigma' z) \sigma^{-1} f'(\lambda) \lambda d\lambda, \quad (5)$$

the paths of integration being the same as in  $\Pi_0$ . With the help of the border conditions the factors are found to be

$$1 + f(\lambda) = (k^2/k'^2)f'(\lambda) = (k^2\sigma' - k'^2\sigma)/(k^2\sigma' + k'^2\sigma). \quad (6)$$

The singularities of the integrands of (4) and (5) are represented in Fig. 1. They consist of the branch points,  $\lambda = k$ ,  $\lambda = k'$ , and of the pole,  $\lambda = k_0$ , of the expression (6). The path of integration ( $L$ ) can be displaced and is equivalent to two loops  $Q_1$  and  $Q_2$  around the branch cuts and to the residuum at the pole. As we mentioned in Section 1, the loops represent the space-waves  $Q = (Q_1 + Q_2)$ , the residuum the surface-wave  $P$ .

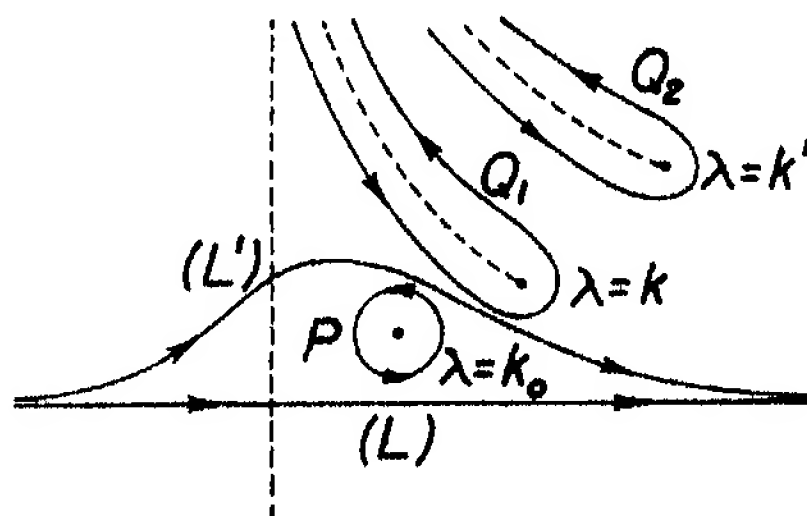


FIGURE 1.

It should be noticed, however, that the integrand of the expression (1) representing the original dipole does not have any singularity at the point  $\lambda = k_0$ . Hence, the path of integration of this integral can be displaced into the curve ( $L'$ ) passing above the point  $\lambda = k_0$ . Since all that is required of the expressions (4) and (5) is that they satisfy the respective wave equations and the border-conditions, the integrals in them can be also conducted over the path ( $L'$ ). Thus, in addition to the  $L$ -solution discussed above we have a second solution which we shall call the  $L'$ -solution. It is given by the integrals (4) and (5) conducted over the path  $L'$ . The evaluation of the  $L'$ -solution can be effected in the same



way, by displacing the path of integration upward. It is thus equivalent to the two loops about the branch cuts (which represent the space waves) and it does *not* include the residuum of the pole (i.e., it does not contain the surface-wave  $P$ ).

The existence of the second solution was heretofore overlooked. Drawing attention to it is the essential contribution of this article. From a mathematical point of view, the existence of two different solutions is not at all surprising because it is well known that the integral of the equation  $\nabla^2 \Pi + k^2 \Pi$ , for given border conditions, is not unique.<sup>6</sup> Since the  $L$ -solution and the  $L'$ -solution each satisfy the differential equations and the border conditions of the problem, it follows that their difference must also satisfy the same conditions and represent a third possible solution. This is given by the integrals (4) and (5) conducted over the paths  $+L$  and  $-L'$ , which are equivalent to a circuit about the pole  $\lambda = k_0$  or to the residuum in this pole. We know already that this residuum represents the surface wave  $P$  of Sommerfeld. We find, in this way, that the surface wave satisfies independently all the conditions of the problem and can exist for itself without any connection with the oscillating pole. This result is not entirely new since in another connection Sommerfeld himself had recognized the independent existence of *surface waves*.<sup>7</sup>

However, Sommerfeld considered there only plane waves while here we have to do with a circular surface-wave. Therefore, it will be well to say a word about this case. The simplest expressions for the  $z$ -components of the Hertzian functions  $\Pi_s$  and  $\Pi_s'$ , (in the two media) of a circular surface-wave are as follows

$$\Pi_s = AH(\lambda r) \exp. (-\sigma z), \quad \Pi_s' = BH(\lambda r) \exp. (\sigma' z), \quad (7)$$

where  $A$  and  $B$  are two constant coefficients; the other components being zero.

The border conditions (3) take then the form

$$k^2 A - k'^2 B = 0, \quad \sigma A + \sigma' B = 0.$$

The two equations are compatible only when their determinant vanishes,

$$k'^2 \sigma + k^2 \sigma' = 0. \quad (8)$$

Hence, the value which the parameter  $\lambda$  must be given in the expressions (7) is the root of the eq. (8). This root,  $\lambda = k_0$ , is identical with the pole of the functions (6). Therefore, it is easy to see that the expressions (7) become identical with the residua of the functions (4) and (5) for the pole  $\lambda = k_0$ , when the constants  $A, B$  are suitably chosen. In other words, they are identical with Sommerfeld's surface wave  $P$ .

From the physical point of view, the most interesting of our results is the existence of the  $L'$ -solution. As this solution contains the oscillating

dipole but not the surface wave, it shows conclusively that the latter wave is not a part of the dipole radiation and is not generated by the dipole. This is true not only for electrodynamic but also for elastic oscillating dipoles which also do not generate (seismic) surface waves. Yet, apart from dipoles, the surface waves can exist and in seismology they are regularly observed in connection with earthquakes. The question how they are generated is an important one but it lies outside the scope of this article.

<sup>1</sup> Sommerfeld, A., *Annalen. Physik*, **28**, 665 (1909).

<sup>2</sup> Weyl, H., *Annalen. Physik*, **60**, 481 (1919).

<sup>3</sup> Sommerfeld, A., contribution to "*Frank-Mises, Differential- und Integral-gleichungen. Zweiter Teil*," p. 932 (Braunschweig, 1935).

<sup>4</sup> Noether, F., *Funktionentheorie und ihre Anwendungen in der Technik*, 165 (1931). The reference was not accessible to the author.

<sup>5</sup> Fu, C. Y., *Geophysics*, **12**, 57 (1947).

<sup>6</sup> Sommerfeld attempted a proof of the uniqueness (reference 1, pp. 680-682). However, this proof breaks down for wave functions which possess source singularities, as both the oscillating dipole and the surface wave do. In this respect the wave equation is in no way different from the Laplacean equation.

<sup>7</sup> Frank-Mises, *loc. cit.*, 877, 878, 930.



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## *FORCES BETWEEN POLYATOMIC MOLECULES*

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Much progress has been made during recent years in understanding the liquid state. Most of it has been based, quite properly, upon the simplest possible model, that of spherically symmetrical molecules, of which the monatomic noble gases furnish the examples. However, most of the molecules with which we have to deal are polyatomic, and we are faced with the question of their conformity to the monatomic model.

The structures of several monatomic liquids have been revealed by x-ray diffraction. This structure is expressed by a radial distribution function which describes the short range order around a given molecule. With it, one can calculate the number of molecular centers within a sphere of any radius,  $r$ , around the reference molecule. We<sup>1</sup> have shown experimentally that this is a general function of the degree of expansion of the liquid over its close-packed structure, and have shown, further,<sup>2</sup> how it could be used to express the relation between the energy of vaporization of a liquid and the intermolecular potential energy by means of a continuous integration, analogous to the summation over all lattice pairs in the case of crystals. The formula is,

$$E_g - E_l = \Delta E_{\text{vap.}} = \frac{-2\pi N^2}{v} \int \epsilon W r^2 dr,$$

where  $E_g$  and  $E_l$  refer to the potential energies per mole in the gas and liquid, respectively,  $N$  is the Avogadro number,  $v$  the molal volume of the liquid,  $W$  the experimental distribution function,  $\epsilon$  the potential energy between pairs of molecules at distances,  $r$ . If we neglect repulsion and express  $\epsilon$  by  $-k/r^6$ , in accord with the theory of London,<sup>3</sup> we can write the approximate equations,

$$E_g - E_l = \Delta E_{\text{vap.}} = \frac{2\pi N^2 k}{v} \int \frac{W dr}{r^4},$$

and

$$\frac{\partial^2 E_l}{\partial v^2} = \frac{2\pi N^2 k}{v^2} \int \frac{W dr}{r^4}$$

Since the integral changes but little with temperature, we can write,

$$v \Delta E_{\text{vap.}} \approx v^2 (\partial E / \partial v) \approx a,$$

a constant corresponding to the van der Waals'  $a$ , though not necessarily having the same numerical value as that computed from critical data. It is a remarkably good one, for experimental values determined from  $\Delta E$  and  $\partial E / \partial v$  agree within a few per cent.

This approach has been extended to give a formula for the energy of mixing two liquids whose molecules, while possessing different radii and fields of force, are nevertheless mixed with maximum randomness by thermal agitation, a kind of solution we have called "regular."<sup>4</sup> The fundamental expression for the energy of mixing  $n_1$  and  $n_2$  molecules in volume  $v$  is,  $(2\pi N^2/v)[n_1^2 \int (11) + n_2^2 \int (22) + 2n_1 n_2 \int (12)]$ , where the integrals are those corresponding to the first equation, above, but with the integrals referring to like and unlike pairs, respectively. An approximate solution of this equation gives, for the partial molal energy of solution of component 1,

$$\bar{E}_1 = \phi_1^2 v_1 [(\Delta E_1/v_1)^{1/2} - (\Delta E_2/v_2)^{1/2}],$$

where  $\phi$  denotes volume fraction, and  $\Delta E_1$  and  $\Delta E_2$  refer to the pure liquid components. For regular solutions, we have, further, the possibility of calculating solubility relations from  $\bar{E}_1$ . I wish to mention that Dr. Scatchard<sup>5</sup> has contributed significantly to these developments.

This formula has proved far more useful than one had any right to expect in view of the approximations that had to be made to put it into practical form. It not only neglects repulsions and possible dipole interaction, but was derived on the assumption of spherical molecules with similar, radial force fields. Nevertheless, it applies remarkably well to polyatomic molecules, provided that they are not too unsymmetrical or different in size. The following illustrations are selected from many.

Vold<sup>6</sup> measured the heat of mixing  $\text{CCl}_4$  and  $\text{SiCl}_4$  and found 32.8 cal. per mole for a 50 mole per cent mixture, while the value calculated from the equation was 28.0 cal.

We predicted that  $\text{I}_2$  and  $\text{CCl}_4$  should give two liquid phases above the melting point of iodine, with a consolute temperature between 150°C. and 170°C. with the mole per cent of iodine about 70. Experiment gave 160.5°C. and 68 mole per cent.

The substances  $\text{SiCl}_4$  and  $\text{SnI}_4$  differ so greatly as to form two liquid phases above the melting point of the latter. From the measured solu-

bility<sup>7</sup> of  $\text{SnI}_4$  in  $\text{SiCl}_4$  at  $59^\circ\text{C}$ . we calculate 117.6 cal./cc. for  $\Delta\epsilon/v$  of  $\text{SnI}_4$  while the heat of vaporization gives 118.3 cal./cc.

But in spite of such successes, there is reason to believe that the field of force around large, polyatomic molecules is not best described as radial. The London forces are very short range, and it is the peripheral atoms which are most important.  $\text{OsF}_8$ , for example, is very volatile although Os is extremely non-volatile. I have sought to throw light upon this question by a rather simple, direct test; a comparison of the entropy of vaporization of two liquids under different but corresponding conditions. My colleague, Dr. Pitzer,<sup>8</sup> has pointed out that two liquids which obey the theory of corresponding states should have equal entropies of vaporization at equal ratios of  $v_g/v_l$ , but that this presupposes, among other conditions, radial force fields of the same shape.

Now there are also two other rules regarding the entropy of vaporization. Trouton's rule, the historic one, states that all normal liquids have the same entropy of evaporation at their boiling points. But this is not strictly true, for there is a gradual increase with increasing boiling point, and I proposed years ago, as a substitute, a rule that has come to be known as the "Hildebrand Rule",<sup>9</sup> according to which the comparison should be made at equal vapor volumes instead of pressures.

Now two van der Waals' liquids should have the same entropy of vaporization not necessarily at equal  $v_g/v_l$  but at equal  $v_g/(v_l - b)$ , as can be seen from the following equations:

$$(\partial P/\partial T)_v = R/(v - b) = (\partial s/\partial v)_T;$$

$$s_g - s_l = R [\ln v_g - \ln(v_l - b)].$$

This follows also from a simple statistical analysis.<sup>10</sup> We can see that for the noble gases there is high probability that  $v_l$  is proportional to  $v_l - b$ , but this is not necessarily the case for larger, polyatomic molecules, where the peripheral atoms are the main centers of attraction. In order to bring to light any significant difference between evaporation at equal  $v_g$  and equal  $v_g/v_l$ , it is necessary to select liquids which differ considerably in liquid volume but which are otherwise as nearly alike as possible. Table 1 shows the comparison between two pairs, first, chlorine and carbon tetrachloride; second, ethane and diisopropyl. Good thermodynamic data are available for our purpose.

TABLE I  
ENTROPY OF VAPORIZATION  
 $\Delta S$  AT SAME

REFERR. LIQUID	T	$\Delta H$	$v_g$	$v_g/v_l$	$T/T_0$	FOR
$\text{Cl}_2$	205	25.3	24.7	27.2	29.7	$\text{CCl}_4$
$\text{Cl}_2$	223	22.6	21.7	24.2	26.3	$\text{CCl}_4$
$\text{C}_2\text{H}_6$	148	25.8	25.5	28.3	31.8	$\text{C}_6\text{H}_{14}$ *
$\text{C}_2\text{H}_6$	160	23.2	22.7	25.5	28.3	$\text{C}_6\text{H}_{14}$

\* Di-isopropyl.



It can be seen that the entropy of evaporation,  $\Delta s$ , for  $\text{CCl}_4$  agrees best with that for  $\text{Cl}_2$  when the comparison is made at equal vapor volumes; less well at equal ratios of vapor to liquid, and much more poorly at equal corresponding temperatures.<sup>11</sup> The agreement with the Hildebrand rule is even closer with ethane and diisopropyl.

It must be concluded, I believe, that the forces between polyatomic molecules are chiefly those between their neighboring parts. Although the assumption of similar radial fields does remarkably well for molecules of moderate size, it will prove increasingly inadequate as one considers larger and larger compact molecules. The localization to which we are accustomed in the case of coulombic forces where they exist in large molecules is even more necessary when considering van der Waals' forces, in view of their much shorter range.<sup>12</sup>

<sup>1</sup> Campbell, J. A., and Hildebrand, J. H., *J. Chem. Phys.*, **11**, 334 (1943).

<sup>2</sup> Hildebrand, J. H., and Wood, S. E., *Ibid.*, **1**, 12 (1933).

<sup>3</sup> London, F., *Trans. Farad. Soc.*, **33**, 8 (1937).

<sup>4</sup> Hildebrand, J. H., "Solubility of Non-electrolytes," Reinhold Pub. Corp. 1936, p. 65ff.

<sup>5</sup> Scatchard, G., *Chem. Rev.*, **8**, 321 (1931).

<sup>6</sup> Vold, R. D., *J. Am. Chem. Soc.*, **59**, 1510 (1937).

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## URETHANE (ETHYL CARBAMATE) THERAPY IN SPONTANEOUS LEUKEMIAS IN MICE\*†

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Palliative effects due to urethane therapy have been reported in human cases.<sup>1, 2</sup> The response to urethane in lymphoid leukemia was less pronounced and more variable than in myeloid leukemia. On the other hand the responses of transplantable animal leukemias (rat and mouse) were found by the same authors<sup>3</sup> to be very much less striking than in humans, if evident at all. Murphy and Sturm<sup>4</sup> have shown a marked effect of urethane on the percentage of "takes" of a transplantable lymphoid leu-

kemia in the rat. Recently, it has been shown in a small series of mice inoculated with a chronic myeloid chloroleukemia that certain palliative effects similar to x-ray therapy were obtained.<sup>5</sup> The effect on prolongation of life was not determined in any of the above experiments.

Preliminary work is reported here relating to the effect of urethane on spontaneous leukemias arising in the C<sub>58</sub> and RIL, subline B, inbred leukemic strains of mice. In this laboratory approximately 90% of mice of both sexes develop leukemia beginning at 6 months of age in the C<sub>58</sub> strain. The majority of these leukemias are lymphoid (lymphocytic) although occasionally myeloid and "stem-cell" leukemias appear. Early symptoms of leukemia in mice of this strain can be detected by periodic palpation of subcutaneous lymph nodes. Usually a single lymph node is initially involved.<sup>6</sup> In all such cases diagnosed in this manner as leukemia, immature leukocytes have been found in the circulating blood and the animal has developed a generalized, systemic disease. Thus, life expectancy within this strain may be determined easily and a moderate degree of accuracy of the effect of chemotherapeutic agents on length of life of leukemic mice may be expected. An incidence of nearly 80% leukemia has been observed in the RIL strain of mice. The incidence is slightly higher among females. Myeloid, lymphoid and "stem-cell" leukemias have been found and the majority of leukemias involve initially and principally the thymus. In these cases the first symptom observed is dyspnea and the animals are at this time in the terminal stages of the disease.

Thirty-eight cases of spontaneous leukemia have been observed in this preliminary study, the majority arising in the C<sub>58</sub> strain.

Urethane<sup>7</sup> was administered intraperitoneally in aqueous solution. The daily dosage was 0.5 mg./gram of body weight. The dose per gram of mouse was contained in 0.0083 cc. distilled water. This sub-anesthetic dose did not materially effect the body weight.

The majority of spontaneous cases reported herein received urethane therapy beginning from 3 to 8 days following discovery of symptoms by palpation. Thus, in many of the mice in the experimental series, therapy was instituted after the disease was well advanced. The effect of urethane was similar in all spontaneous cases studied. After 3 daily doses (approximately 45 mg. urethane) white blood-cell counts dropped markedly. In most cases white blood-cells counts were down to normal levels within 7 days and with continued therapy the counts remained within normal limits or the animals developed a definite leukopenia. Only 2 cases were observed in which during the terminal stages of the disease (day of death or day preceding death) the white blood-cell count returned to extremely high levels.

Differential white-cell counts obtained periodically following treatment of spontaneous cases showed a drop in percentage of immature cells. In

TABLE 1—TYPICAL BLOOD PICTURES IN SPONTANEOUS FOLLOWING URETHANE TREATMENT

ANIMAL	AGE, MO.	TREATMENT BEGUN AFTER SYMPTOMS, DAYS	BLOOD COUNTS									
			INITIAL									
			TOTAL WBC	LYMPHOCYTES, %	POLYMORPHS, %	IMMATURES, %	HB, G./100 CC.	TOTAL WBC	LYMPHOCYTES, %	POLYMORPHS, %	IMMATURES, %	HB, G./100 CC.
♀ C <sub>58</sub> 38†	8	4	43,375	12	46	41	13.5	14,800	24	40	30	11.5
♀ C <sub>58</sub> 80307	14	7	144,750	66	5	28	14.5	36,250	57	11	30	11.5
♂ RIL 28	8	8	20,000	32	47	21	12.0	11,625	8	72	20	11.5
♀ C <sub>58</sub> 78977	15	4	21,750	42	24	34	11.0	34,875	47	18	34	11.0
♂ C <sub>58</sub> 79508	15	6	24,250	37	20	42	11.0	8,000	36	30	30	11.0
♀ C <sub>58</sub> 62	7	5	36,625	70	17	13	15.5	6,750	55	38	6	14.5
♂ C <sub>58</sub> 71	8	5	13,875	44	37	19	13.0	3,850	31	56	8	13.0
Averages			44,803	43.3	28	28.3	13.3	15,878	37	38	22.0	12.0
♀ C <sub>58</sub> 15	11	None	14,000	42	28	30	15.5	26,375	39	26	32	8.0
♂ C <sub>58</sub> 40	9	None	38,250	53	23	19	15.0	32,375	45	9	41	13.0
♂ RIL 17	10	None	68,000	6	28	64	11.0	120,000	3	36	60	9.5
♀ C <sub>58</sub> 79	6	None	38,725	55	11	33		36,500	34	11	53	
♀ C <sub>58</sub> 67†	6	None	17,875	34	49	17		7,875	18	72	8	12.5
								36,500			13	

\* First reading following urethane therapy at 3 days. Subsequent readings at approximately 7-day intervals.

most cases this was a gradual decrease with continued therapy resulting in levels of only a few immature cells at approximately 9 to 10 days. These levels usually remained low until death. Two cases of myeloid leukemia in the C<sub>58</sub> strain responded in the same manner. The amount of reduction was found not to be proportional to the pre-treatment immature cell count.

In addition to the marked decrease in total white blood cells and immature forms there resulted also a conspicuous decrease in circulating lymphocytes and a corresponding increase in polymorphonuclear leukocytes. This phenomenon was described by Hawkins and Murphy<sup>8</sup> in the blood of rabbits subjected to urethane anesthesia.

Following is a typical example of response of a spontaneous lymphoid leukemia to the drug: C<sub>58</sub> ♂ 79508 was administered urethane, 15 mg. daily, five days after appearance of symptoms. The white-cell count had fallen to 12% of the original figure after 7 days of treatment. This leukopenic level was maintained by therapy throughout the life of the animal. The immature cells showed a reduction in absolute count to 25% of the original after 13 days and to 4.8% (2% immature cells) of the original after 18 days of treatment. This level was maintained by therapy. The typical decrease in circulating lymphocytes and increase in polymorphonuclear

LEUKEMIC MICE OF THE C<sub>58</sub> AND RIL STRAINS  
(SUB-ANESTHETIC DOSE)

FOLLOWING URETHANE THERAPY\*

POLYMORPHS, %	IMMATURES, %	HB, G./100 CC.	TOTAL WBC	LYMPHOCYTES, %	POLYMORPHS, %	IMMATURES, %	HB, G./100 CC.	TOTAL WBC	LYMPHOCYTES, %	POLYMORPHS, %	IMMATURES, %	HB, G./100 CC.	TOTAL WBC	LYMPHOCYTES, %	POLYMORPHS, %	IMMATURES, %	HB, G./100 CC.
41	16	8.0	9,750	16	74	10	8.0	4,975	12	64	24	8.0	7750	4	92	4	8.0
28	9	11.5	8,125	48	28	22	11.0	11,875	30	57	11	11.0					
72	14	9.0	5,125	18	78	4	5.3										
36	15	9.5	8,000	21	56	23	12.5	9,625	22	53	22	10.5	8625	41	38	21	8.0
65	11	9.3	7,500	31	67	2	10.5	4,250	8	84	2	8.0					
35	6	14.5	11,825	38	58	4	11.5	39,750	25	71	4	7.7					
60	12	11.5	6,000	16	82	2	11.0										
49	11.8	10.5	8,046	27	63.3	9.6	9.9	14,095	19.4	66	12.6	8.4					
24	50	7.0															
12	44	13.5	107,000	27	16	57	11.5										
33	54	10.5															

† Myeloid leukemia. All others lymphoid.

‡ Urethane therapy started in terminal stages 12 days after appearance of symptoms.

leukocytes was evident after 7 days of treatment. After 27 days the circulating lymphocytes had fallen from 37 to 8% and the polymorphonuclear leukocytes had increased from 20 to 84%.

In contrast to the results obtained in human cases<sup>1</sup> there was not observed a general rise in hemoglobin levels. In 2 treated mice only was there observed a rise in hemoglobin levels. In ♀ C<sub>58</sub> 78977 the hemoglobin value rose by an average of 10.7% (100% = 14.5 g. Hb per 100 cc.) after 19 days of therapy. In ♂ C<sub>58</sub> 81 the hemoglobin value rose by an average of 11.1% after 17 days of treatment. Indications are, however, that the fall in hemoglobin levels in urethane treated leukemias is not so precipitous as in the untreated controls. This is more evident in spontaneous cases given urethane immediately upon discovery of symptoms. These cases are not included in this series in which hemoglobin levels were relatively low when therapy was initiated. (See table 1 for typical response of blood counts in spontaneous cases of leukemia.)

Marked changes in the blood picture were evident 24 hours after administration of urethane (sub-anesthetic dose). The leukemic blood picture returned immediately upon cessation of treatment.

In mice given urethane within 3 to 5 days after discovery of palpable nodes, the subcutaneous node (or nodes) regressed completely within 2 to

3 days of continued therapy. In cases where urethane was given 8 days or later after appearance of symptoms a definite decrease in size resulted. The difference in mean weights of subcutaneous lymph nodes (axillary, inguinal and cervical) in the control and urethane-treated cases was 248.8 mg. where  $t = 2.7$  and  $P = 0.02\%$ .

There was a definite decrease in the size of the spleen in spontaneous cases already showing splenic involvement upon therapy. In cases where treatment was begun before the spleen was palpable the spleen did not become infiltrated greatly. The difference in mean weights of the spleen in control and urethane mice obtained at autopsy was 324.0 mg. where  $t = 1.29$  and  $P < 0.3\%$ . There was considerable variation in splenic weights in the urethane-treated series probably as a result of institution of therapy at various time intervals after appearance of symptoms.

TABLE 2  
COMPARISON OF MEAN WEIGHTS OF SPLEEN, LYMPH NODES AND THYMUS IN URETHANE-TREATED AND CONTROL SPONTANEOUS LEUKEMIAS

	NO.	WEIGHTS IN MILLIGRAMS		
		SPLEEN	LYMPH NODES*	THYMUS†
Urethane-treated	7	731.4	194.3	73.7
Controls	13	1055.4	479.1	530.8
Difference (means) =		324.0	284.8	457.1
$t =$		1.29	2.7	3.7
$P =$		< 0.3%	0.02%	< 0.01%

\* Including all axillary, inguinal and cervical lymph nodes. All weights obtained at death of animals.

† Thymus weights on 8 experimental and 13 control animals were obtained principally from RIL, subline B, pedigreed mice and are unrelated to spleen and lymph node weights obtained principally from C<sub>58</sub> pedigreed mice with systemic disease originating in subcutaneous lymph nodes.

The mean length of life of untreated spontaneous leukemias in the C<sub>58</sub> strain was  $21.8 \pm 7.15$  days and in urethane-treated leukemias  $35.9 \pm 8.76$  days following discovery of symptoms by palpation. In one case, ♀ C<sub>58</sub> 80302 life was maintained for 52 days. Although the difference of the means is not significant in the series reported here, indications are that if urethane is given early, following discovery of symptoms, a greater life expectancy may be obtained.

Leukemic mice from both the C<sub>58</sub> and RIL strains (8 animals), in which there was initial thymic involvement resulting in dyspnea followed usually by a rapid, generalized systemic course of the disease, were given urethane treatment immediately upon discovery of the dyspnea. There resulted a marked depression in the white-cell count, a decrease of immatures in the circulating blood and in most cases a sudden disappearance of dyspnea. In these mice there was a significant increase in the length of life,  $22.7 \pm 3.1$  days compared with  $7.7 \pm 5.34$  days, for untreated controls. At



autopsy it was found that the thymus had regressed, was soft, spongy and yellowish-brown in color. The difference in mean thymus weights between the urethane-treated and control series was 457.1 mg. where  $t = 3.7$  and  $P < 0.01\%$  (see table 2).

The following histological picture obtains in leukemic mice receiving daily sub-anesthetic doses of urethane: In the lymph nodes of mice receiving urethane shortly after appearance of symptoms there was observed in most cases an intense necrobiosis with pyknosis, karyorrhexis and chromatolysis. This was usually generalized throughout the nodes. However, the process was localized in leukemic mice in which therapy was begun in the terminal stages of the disease. Where therapy had been continued for a relatively long period of time there were found areas of necrosis in the lymph nodes. In several cases moderate to intense reticulosis and fibrosis were observed. The reactions of splenic tissue to urethane were similar but not so intense as in lymph nodes.

The most pronounced effect of the drug has been observed in the thymus where intense generalized necrobiosis, large areas of necrosis and fibrosis and severe hyperemia were observed.

Phagocytosis was very prominent in the livers of treated mice. Large areas of perivascular fibrosis in regions of leukemic infiltration were present. In some hepatic cells there was a moderate degree of vacuolar degeneration. Hyperemia was pronounced in some livers.

In ♂ RIL 28 in which there was severe thymic involvement and in which urethane therapy was started 8 days after appearance of symptoms there occurred moderate leukemic infiltration in the lung. There was observed in these areas of infiltration a very intense necrobiosis.

More complete details concerning the histology of urethane-treated tissues will be given later.

*Summary.*—Definite palliative effects of daily sub-anesthetic doses of urethane have been observed in 18 cases of spontaneous leukemia in the C<sub>58</sub> and RIL inbred strains of mice. The majority of these generalized systemic leukemias were lymphoid although the response of two myeloid leukemias was similar. The effects produced were: (1) a pronounced fall in total white-cell count to or below normal limits and a maintenance of these levels with continued urethane therapy; (2) a marked reduction in the number of immature cells in the circulating blood; (3) a possible stabilizing effect on the hemoglobin levels; and (4) a pronounced diminution in the size of enlarged subcutaneous lymph nodes and spleen. The life expectancy of urethane-treated leukemics is greater than control leukemics but not of statistical significance.

In a series of 8 leukemic mice of the RIL strain in which there was initial and principal thymic involvement the same palliative effects were obtained. In addition there was relief from symptoms of dyspnea and tho-



racic enlargement, a statistically significant decrease in the size of the thymus, and a statistically significant lengthening of life.

A generalized necrobiosis in the spleen, thymus and lymph nodes followed by localized necrosis in animals receiving long continued therapy has been observed. Moderate to intense reticulosis and fibrosis were evident in cases receiving treatment early and over a relatively long period of time.

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† Technical assistance of Mr. Lester E. Bunker, Jr., and Miss Betty-Ann Norris is gratefully acknowledged.

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<sup>6</sup> Biopsy samples of lymphatic tissue of mice of the C<sub>58</sub> strain, taken at regular intervals by Potter, Victor and Ward indicated that reticulum cell hyperplasia which these authors interpret as sites for primary malignant lymphocytopoiesis, occurred usually in only one or two subcutaneous nodes and that invasion accounted for the widespread lesions as the disease progressed. See Potter, J. S., Victor, J., and Ward, E. N., *Am. Jour. Pathology*, **19**, 239-249 (1943).

<sup>7</sup> The urethane used in this preliminary work was supplied through the generosity of Prof. Alexander Haddow.

<sup>8</sup> Hawkins, J. A., and Murphy, J. B., *Jour. Exp. Med.*, **42**, 609-618 (1925).

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## INFLUENCE OF HUMIDITY ON THE SURVIVAL OF DIFFERENT CHROMOSOMAL TYPES IN *DROSOPHILA PSEUDOOBSCURA*

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Populations of *Drosophila pseudoobscura* show a high variability with respect to the gene arrangement in the third chromosome. Several gene arrangements, which must have arisen from each other by inversions of blocks of genes, occur frequently in the same territory. Populations of different localities very often differ in the relative frequencies of the gene arrangements,<sup>1</sup> and in at least some localities, the frequencies change also from month to month, the changes being connected with the succession of year's seasons. In particular, seasonal changes are observed in the populations of Piñon Flats and Andreas Canyon, on Mount San Jacinto in California. The Standard gene arrangement increases in frequency during

the hot period of the summer, remains rather constant during autumn and winter, and decreases in frequency during spring. The Chiricahua arrangement undergoes a cycle opposite in sign to that of Standard, and the Arrowhead arrangement shows relatively little change.<sup>2</sup> These data suggest that carriers of different gene arrangements differ in adaptive value, and that the seasonal changes represent adaptive responses of the populations to changing conditions of the milieu. Artificial populations kept in specially constructed "population cages" bear out the hypothesis of natural selection, in so far as, at relatively high temperatures (above 21°), changes are induced which seem to parallel those observed in natural habitats in summer. Thus, if the initial population of a population cage contains less than 50 per cent of Standard and more than 50 per cent of Chiricahua chromosomes, the former increase, and the latter decrease in frequency, until an equilibrium is established at about 70 per cent Standard and 30 per cent Chiricahua. Similarly, Standard is, at high temperatures, relatively superior to Arrowhead, and Arrowhead is superior to Chiricahua. At temperatures below 21°, the adaptive values of the carriers of all gene arrangements appear to be alike.<sup>3</sup>

The experiments just referred to describe, however, the net differences between adaptive values of the different gene arrangements. It is not known at just what stages of the developmental cycle the differential survival takes place. Furthermore, in the experiments so far published, the carriers of the Chiricahua gene arrangement have never proved superior in adaptive value to those with Standard and Arrowhead. Yet, in the natural populations, Chiricahua chromosomes do increase in frequency at the expense of the others during the spring season. It follows that, an ecological niche should be found in which the carriers of Chiricahua will be relatively superior. One of the environmental agents never tried before which may have an effect on the survival of the chromosomal types, is humidity. The pupal stage is especially likely to be sensitive to humidity variations. Among the known facts which point in this direction, suffice it to mention that, differential mortality of wild type pupae and pupae homozygous for certain mutants has been found in *Drosophila melanogaster* at low humidities.<sup>4</sup>

In the experiments to be described below, *Drosophila pseudoobscura* pupae, homozygous for Standard, Arrowhead, and Chiricahua gene arrangements have been used. The ancestors of all the experimental flies came from the Piñon Flats locality in California. At least ten strains with each gene arrangement were intercrossed, so that the experimental flies, though structurally homozygous, were genically heterozygous (the importance of which is discussed by Wright and Dobzhansky<sup>5</sup>). The parental flies were placed in population cages, some hundreds of males and females of each type in a different one. The cages were kept in a constant temperature

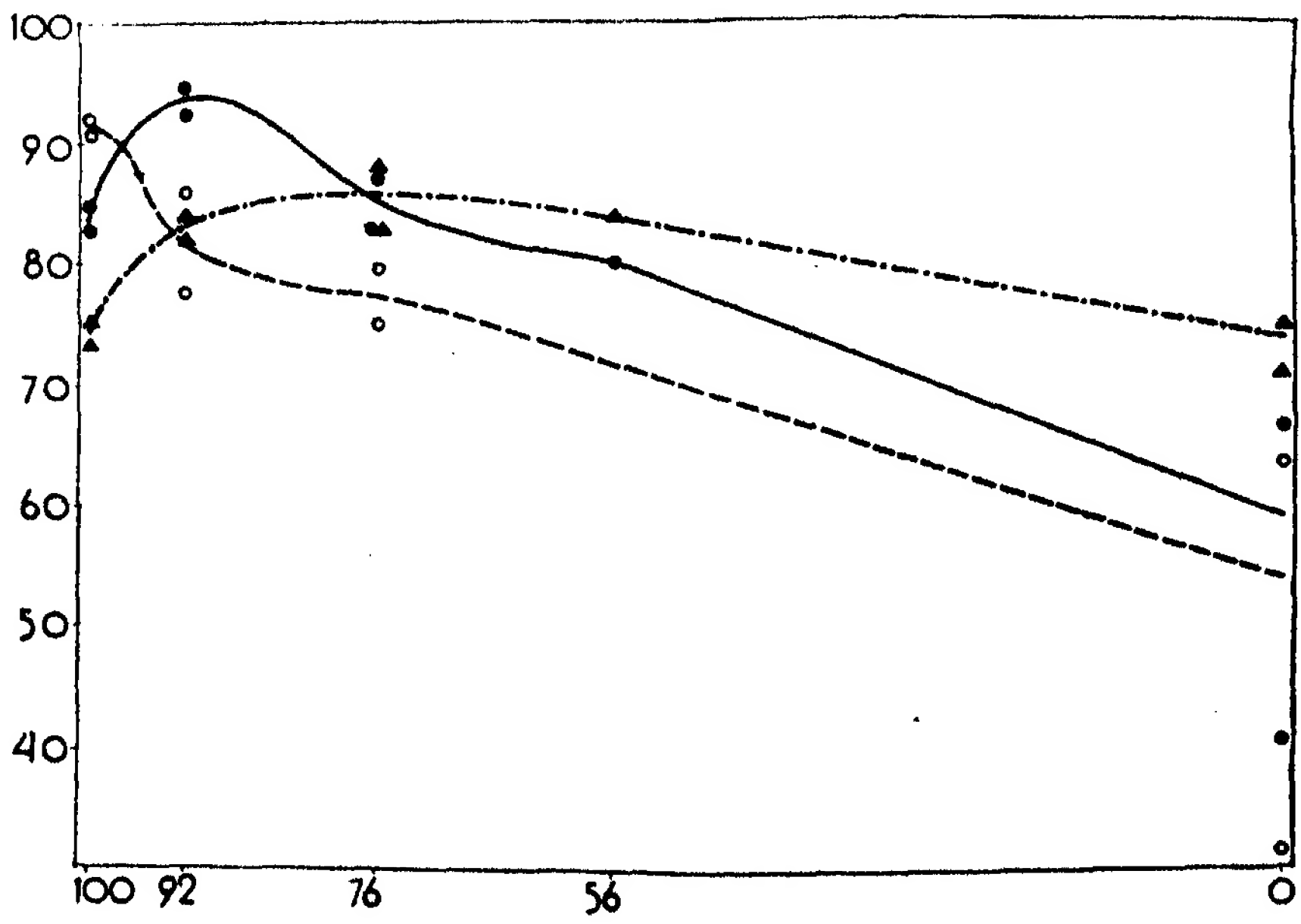


FIGURE 1

Ordinates: percentages of hatched pupae. Abscissae: relative humidity in per cent. Solid circles—Standard pupae; open circles—Chiricahua pupae; triangles—Arrowhead pupae.

TABLE I

HUMIDITY	GENO- TYPE	FIRST EXPERIMENT			SECOND EXPERIMENT			TOTAL		
		PUPAE	FLIES	%	PUPAE	FLIES	%	PUPAE	FLIES	%
100%	ST/ST	300	248	82.6	400	340	85.0	700	588	84.0
100%	CH/CH	300	276	92.0	400	363	90.7	700	639	91.0
100%	AR/AR	300	220	73.3	400	300	75.0	700	520	74.5
92%	ST/ST	300	278	92.6	300	284	94.6	600	562	93.6
92%	CH/CH	300	233	77.6	300	258	86.0	600	491	81.8
92%	AR/AR	300	256	82.0	300	252	84.0	600	508	84.6
76%	ST/ST	500	419	83.2	300	262	87.3	800	681	85.1
76%	CH/CH	500	376	75.2	300	239	79.2	800	615	76.9
76%	AR/AR	300	265	88.3	300	250	83.3	600	511	85.1
56%	ST/ST	..	..	..	300	242	80.6	300	242	80.6
56%	AR/AR	..	..	..	200	168	84.0	200	168	84.0
0%	ST/ST	300	122	40.8	700	468	66.9	1000	590	59.0
0%	CH/CH	300	94	31.3	700	446	63.7	1000	540	54.0
0%	AR/AR	300	215	71.0	700	526	75.1	1000	741	74.1

room at 19°, and at relative humidities ranging from 45 to 50 per cent. When pupae began to form in the cages, they were extracted individually by means of a needle, and transferred into glass vials, one hundred pupae per vial. The vials were closed by cheese cloth held by a rubber band.

Only very young and light pupae were taken. The vials were placed in desiccators at the desired humidities. Five relative humidities were used, namely 100%, 92%, 76%, 56% and 0%. They were obtained by placing on the bottom of the desiccators, distilled water,  $K_2SO_4$ , NaCl, NaBr in oversaturated solutions, and anhydrous  $CaCl_2$ , respectively (according to Ludwig and Landsman<sup>4</sup>). The desiccators with pupae were placed at a constant temperature of 25°C.

The experiments were replicated twice. The results obtained are reported in table 1, and represented graphically in figure 1. It can be seen clearly that, at the temperature of 25°C., Chiricahua pupae are more viable than Standard, and the latter more viable than Arrowhead at 100% humidity. At 92% humidity, Standard is superior to the other two. At 76%, Standard and Arrowhead are alike, but both of them are superior to Chiricahua. At lower humidities, Arrowhead is superior to both Standard and Chiricahua. Thus, Chiricahua is most favorable, and Arrowhead least favorable, at 100% humidity, but the relations are reversed at 0%. The two series of experiments showed the same hierarchy of hatchabilities of the pupae with the three gene arrangements, although in the first experiment the hatchabilities were lower in almost all humidities than they were in the second experiment. The difference must have been due to more favorable environmental conditions in the second than in the first series of population cages in which the pupae were reared.

The results of the experiments just reported suggest that, flies with Chiricahua chromosomes are relatively better adapted to climates with high humidities, and Arrowhead flies to low humidities, Standard being intermediate. The temporary superiority of Chiricahua observed during the spring season at Piñon Flats might, then, be due to the relatively high humidities prevailing at that season. The validity of this hypothesis must, of course, be tested by studying the survival rates of pupae at different temperatures.

I wish to express my gratitude to Prof. Th. Dobzhansky for his generous hospitality throughout the course of this investigation, and for his continuous advice and encouragement.

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# PERTURBATIONS OF DISCONTINUOUS SOLUTIONS OF NON-LINEAR SYSTEMS OF DIFFERENTIAL EQUATIONS

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Theorems about solutions of the system

$$\frac{dx_i}{dt} = X_i(x_1, \dots, x_n, t, \epsilon), \quad i = 1, \dots, n, \quad (1)$$

where the  $X_i$  are regular functions of  $\epsilon$  for small  $|\epsilon|$ , are classical. More recently studies of cases of (1) subject to the various conditions that allow one or several  $X_i$  to become large as  $\epsilon \rightarrow 0$  have been studied.<sup>1</sup>

In all the studies just referred to it is assumed that the system (1) has a solution with a continuous derivative in case  $\epsilon = 0$ ; sufficient conditions are given for this also to be the case for  $\epsilon \neq 0$ . There are cases of considerable interest where, when  $\epsilon = 0$ , the system (1) has only a discontinuous "solution." It is known as a practical matter that in these cases when  $\epsilon \neq 0$  but small the system may have a continuous solution which approaches the discontinuous one as  $\epsilon \rightarrow 0$ . Practical applications of this have been made by the Russian school of non-linear mechanics (Mandelstam, Chaikin, Lochakow). A rigorous treatment of this case appears so far to have been given<sup>2</sup> only for a special relaxation oscillation problem involving two unknown functions (so that the simple topology of the phase plane could be exploited).

Here the following system of arbitrary order is considered:

$$\begin{aligned} \frac{dx_i}{dt} &= f_i \frac{du}{dt} + \phi_i, \quad i = 1, 2, \dots, n, \\ \epsilon \frac{d^2u}{dt^2} + g \frac{du}{dt} + h &= 0. \end{aligned} \quad (2)$$

In (2),  $f_i$ ,  $\phi_i$ ,  $g$  and  $h$  are all functions of  $x_1, \dots, x_n, u$ , and  $t$  which we shall denote hereafter by  $f_i(x, u, t)$ , etc. We shall first show that (2) is general enough to include certain cases of obvious interest:

*Example 1.* The van der Pol equation, with a change of time scale, becomes

$$\epsilon \frac{d^2u}{dt^2} + (u^2 - 1) \frac{du}{dt} + u = 0;$$

*Example 2.* The Rayleigh type equation,

$$\epsilon \ddot{x} + (\dot{x}^2 - 1)\dot{x} + x + x^3 = 0,$$

is not included in (2). However if we differentiate it and set  $\dot{x} = u$  we get

$$\frac{dx}{dt} = u,$$

$$\epsilon \frac{d^2u}{dt^2} + (3u^2 - 1) \frac{du}{dt} + u + 3x^2u = 0$$

which is of the form (2);

*Example 3.* Consider the system<sup>3</sup>

$$\frac{dx_i}{dt} = H_i(x, w, t), \quad i = 1, \dots, n,$$

$$\epsilon \frac{dw}{dt} = G(x, w, t).$$

In case the right members above are not linear in  $w$ , this system can be brought to the form (2) simply by differentiating the last equation only with respect to  $t$ .

In case the right members are linear in  $w$ , we can introduce  $u$  defined by  $\dot{u} = w$  and the system assumes the form

$$\frac{dx_i}{dt} = f_i(x, t) \frac{du}{dt} + \phi_i(x, t),$$

$$\epsilon \frac{d^2u}{dt^2} = g(x, t) \frac{du}{dt} + h(x, t),$$

which is a special case of (2).

To formulate our result precisely we consider along with (2) where  $\epsilon > 0$ , the degenerate system

$$\frac{dy_i}{dt} = f_i(y, v, t) \frac{dv}{dt} + \phi_i(y, v, t), \quad i = 1, 2, \dots, n,$$

$$g(y, v, t) \frac{dv}{dt} + h(y, v, t) = 0, \tag{3}$$

which is (2) with  $\epsilon = 0$ . We shall regard a solution of (3) as a curve  $C_0$  in  $E_{n+2}$ , the  $n + 2$  dimensional space  $(y, v, t)$ . We shall assume that there is an open continuum  $D$  in  $E_{n+2}$  which contains the solution  $C_0$  and in which  $f_i$ ,  $\phi_i$ ,  $g$  and  $h$  are continuous and have continuous first order partial derivatives with respect to  $y_i$ ,  $v$  and  $t$ .

*Definition.* The  $n + 1$  functions  $y_i(t)$ ,  $v(t)$ , denoted briefly by the  $n + 1$  dimensional vector  $Y(t)$ , is said to be a solution of (2) the degenerate system (3) in the interval  $\alpha \leq t \leq \beta$  if:



(1.1)  $Y(t)$  is continuous and possesses a continuous derivative except possibly at a finite number of interior points,  $\tau_j$ , of the closed interval  $(\alpha, \beta)$  and except at the  $\tau_j$ ,  $Y(t) = (y_i(t), v(t))$  satisfies (3).

(1.2) Except at the points  $\tau_j$ ,

$$g(y(t), v(t), t) \neq 0.$$

(1.3) Both  $Y(\tau_j - 0)$  and  $Y(\tau_j + 0)$  exist, and we denote them by  $Y_-$  and  $Y_+$ , respectively. Similarly we define  $y_-$ ,  $v_-$ , etc. By  $f_{i-}$  we mean  $f_i(y_-, v_-, \tau_j)$ , etc.

(1.4) For each  $j$ ,  $g_- = 0$ ;

(1.5) For each  $j$ ,  $g_+ \neq 0$ ;

(1.6) Denoting by  $y(v, \tau_j)$  the solution,  $y_i$ , of

$$\frac{dy_i}{dv} = f_i(y, v, \tau_j)$$

for which  $y(v_-, \tau_j) = y_-$  and  $y(v_+, \tau_j) = y_+$ , let

$$\int_{v_-}^{v_+} g(y(v, \tau_j), v, \tau_j) dv = 0.$$

(1.7) There exists no  $v_0$  interior to the interval  $(v_-, v_+)$  such that the integral in (1.6) vanishes if  $v_+$  is replaced by  $v_0$  in the upper limit of the integral.

(1.8) Let  $I$  denote  $\frac{\partial g}{\partial v} + \sum_{i=1}^n \frac{\partial g}{\partial y_i} f_i$ . Replacing  $y$  by  $y_-$ ,  $v$  by  $v_-$  and  $t$  by  $\tau_j$ ,

$I$  becomes  $I_-$ . We assume that

$$(I_-)(h_-) > 0.$$

(1.9) For small  $\delta > 0$ ,

$$g(x(\tau_j - \delta), v(\tau_j - \delta), \tau_j - \delta) > 0.$$

The curve  $C_0$  in  $E_{n+2}$  is determined by  $Y(t)$  for  $t \neq \tau_j$  and by  $y(v, \tau_j)$  where  $v$  goes from  $v_-$  to  $v_+$  when  $t = \tau_j$ . Thus  $C_0$  is continuous, and  $C_0$  possesses a tangent except at the several points  $Y_-$  and  $Y_+$ .

The above rather complicated definition of a discontinuous solution of (1.3) is justified by the following result.

**THEOREM I.** *Let the degenerate system (3) have a solution  $C_0$ , for  $\alpha \leq t \leq \beta$ . Let  $X(t) = (x(t), u(t))$  be a solution of (2) for  $\epsilon > 0$  such that*

$$|X(\alpha) - Y(\alpha)| = \sum_{i=1}^n |x_i(\alpha) - y_i(\alpha)| + |u(\alpha) - v(\alpha)| \leq \delta_1$$

$$\left| \frac{du(\alpha)}{dt} - \frac{dv(\alpha)}{dt} \right| \leq \frac{\delta_2}{\epsilon}$$

If  $\epsilon$ ,  $\delta_1$  and  $\delta_2$  are sufficiently small,  $X(t)$  is a solution of (2) over  $\alpha \leq t \leq \beta$ . Moreover as  $\epsilon$ ,  $\delta_1$  and  $\delta_2 \rightarrow 0$ , the curve representing  $X(t)$  in  $E_{n+2}$  approaches  $C_0$ .

In particular  $X(\beta) \rightarrow Y(\beta)$ . Also  $\frac{du(\beta)}{dt} \rightarrow \frac{dv(\beta)}{dt}$ .

Thus we see that the existence of a solution, even though discontinuous, of the degenerate system (3) implies the existence of nearby solutions of the system (2).

Let us denote the initial values of one of the  $n + 1$  coördinates of  $X(\alpha)$  and  $Y(\alpha)$  by  $a$ . Then we can regard the values of  $X$  and  $Y$  at  $t = \beta$  as functions of  $\beta$  and  $a$ . We have

THEOREM II. As  $\epsilon$ ,  $\delta_1$  and  $\delta_2 \rightarrow 0$ ,

$$\frac{\partial X(\beta, a)}{\partial a} \rightarrow \frac{\partial Y(\beta, a)}{\partial a}.$$

Also as  $\epsilon$ ,  $\delta_1$ ,  $\delta_2 \rightarrow 0$ ,

$$\frac{\partial}{\partial a} \frac{du(\beta, a)}{dt} \rightarrow \frac{\partial}{\partial a} \frac{dv(\beta, a)}{dt}$$

Moreover denoting the value of  $\frac{du}{dt}$  at  $\alpha$  by  $c$ , we have as  $\epsilon \rightarrow 0$

$$\frac{\partial X(\beta, c)}{\partial c} \rightarrow 0, \quad \frac{\partial}{\partial c} \frac{du(\beta, c)}{dt} \rightarrow 0.$$

In case the degenerate system (3) has a periodic solution and the Jacobian associated with the determination of this solution by varying initial conditions is distinct from zero, then it follows from Theorem II that the corresponding Jacobian for (2) also will not vanish and therefore (2) will also have a periodic solution.<sup>4</sup>

The treatment can be generalized readily to the case where not one but several or even all the equations of (2) assume the same form as the last ( $n + 1$ st) equation and moreover where the terms  $f$ ,  $\phi$ ,  $g$  and  $h$  depend on  $\epsilon$  (but approach finite limiting values as  $\epsilon \rightarrow 0$ ).

A further generalization is the following system:

$$\begin{aligned} \frac{dx_i}{dt} &= \sum_{j=1}^m f_{ij}(x, u, t, \epsilon) \frac{du_j}{dt} + \phi_i(x, u, t, \epsilon), \quad i = 1, \dots, n, \\ \epsilon \frac{d^2 u_i}{dt^2} + \sum_{j=1}^m g_{ij}(x, u, t, \epsilon) \frac{du_j}{dt} + h_i(x, u, t, \epsilon) &= 0, \quad i = 1, \dots, m. \end{aligned}$$

Let us denote the square matrix

$$(g_{ij}(x, u, t, \epsilon))$$

by  $G$  and its determinant by  $|G|$ . We assume that the degenerate system ( $\epsilon = 0$ ) has a solution which can be continued up to  $t = \tau$  where  $\tau$  is such that for the solution in question  $|G(x(\tau), u(\tau), \tau, 0)| = 0$ . Part of our sufficient condition for the perturbed system to have a solution is the requirement that the characteristic equation

$$|g_{ij}(x(\tau), u(\tau), \tau, 0) - \lambda \delta_{ij}| = 0$$

have  $\lambda = 0$  as a simple root.

<sup>1</sup> M. Nagumo, Über das Verhalten der Integrals von  $\lambda y'' + f(x, y, y', \lambda) = 0$  für  $\lambda \rightarrow 0$ . *Proc. Phys. Math. Soc. Japan*, **21**, 529–534 (1939). I. M. Volk, A Generalization of the Method of Small Parameter in the Theory of Non-Linear Oscillations of Non-Autonomous Systems. *C. R. (Doklady) Acad. Sci. U.S.S.R.*, **51**, 437–440 (1946). Volk considers (1) where the  $X_i$  are meromorphic functions of  $\epsilon$  for small  $\epsilon$  and periodic in  $t$ . K. O. Friedrichs and W. R. Wasow, Singular Perturbations of Non-Linear Oscillations. *Duke Math. Jour.*, **13**, 367–381 (1946). Here the  $X_i$  are not functions of  $t$  and for  $i \leq n-1$  are not functions of  $\epsilon$ .  $X_n$  contains  $\epsilon$  in the form of a factor  $1/\epsilon$ .

<sup>2</sup> D. A. Flanders and J. J. Stoker, *The Limit Case of Relaxation Oscillations, Studies in the Linear Vibration Theory*, New York Univ., 1946.

<sup>3</sup> This is the system, except that  $t$  is not necessarily excluded from the right members, which is considered by Friedrichs and Wasow, *loc. cit.*

<sup>4</sup> See Friedrichs and Wasow, and Volk, *loc. cit.*, for continuous cases where right members do not and do, respectively, depend on  $t$ .

## A MINIMUM PROBLEM ABOUT THE MOTION OF A SOLID THROUGH A FLUID

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1. An incompressible frictionless fluid of uniform density  $\rho$  fills the whole space outside a moving solid and is at rest at infinite distance. The motion of the solid is one of pure translation. The magnitude of the velocity is  $U$ , its direction cosines with respect to a coördinate system fixed in the solid  $\lambda, \mu, \nu$ . The kinetic energy of the fluid is of the form

$$T = \frac{1}{2}MU^2.$$

The quantity  $M$ , called the virtual mass, depends on the direction of the velocity:

$$M/\rho = A\lambda^2 + B\mu^2 + C\nu^2 + 2A'\mu\nu + 2B'\nu\lambda + 2C'\lambda\mu.$$

$A, B, C, A', B', C'$  are uniquely determined if the shape and size of the solid and the relative location of the coördinate system and the solid are given.

A closer study of the dependence of  $A, B, C, A', B'$  and  $C'$  on geometric data may seem desirable.<sup>1</sup> Taking a first step in such a study, we consider the *average virtual mass*  $\bar{M}$ , obtained by averaging  $M$  over all directions  $\lambda, \mu, \nu$  and assuming  $\rho = 1$ :

$$\bar{M} = (A + B + C)/3.$$

$\bar{M}$  is independent of the location of the coördinate system and depends only on the size and shape of the solid. It is easy to show that *of all ellipsoids with given volume the sphere has the minimum average virtual mass*. It would be natural to suspect that this statement remains true if for "ellipsoids" we substitute "solids." At any rate, I shall prove the analogous general theorem in two dimensions.

2. We consider now the two-dimensional motion of an incompressible frictionless fluid of uniform density  $\rho$  that fills the space around a cylinder of infinite length. The motion is parallel to a plane, the plane of the complex variable  $z$ , that is perpendicular to the cylinder and intersects it in a closed curve  $C$  (the notation of section 1 has been dropped). The exterior of  $C$  is mapped conformally onto the exterior of the unit circle in the  $\zeta$ -plane so that the points at infinity correspond. Thus,  $z$  moving outside  $C$  is represented by the series

$$z = \lambda \left( \zeta + c_0 + \frac{c_1}{\zeta} + \frac{c_2}{\zeta^2} + \dots \right) \quad (1)$$

convergent for  $|\zeta| > 1$ . The number  $\lambda$  is positive.

We begin with the case in which the motion of the fluid at infinite distance is parallel to the real axis and has the velocity  $U$  (uniform flow disturbed by a fixed cylindrical obstacle). The corresponding motion in the  $\zeta$ -plane, around a circular cylinder and with velocity  $U\lambda$  at infinity, has the complex potential

$$\chi' = U\lambda \left( \zeta + \frac{1}{\zeta} \right). \quad (2)$$

Yet (2) represents also the complex potential for the  $z$ -plane provided that  $z$  and  $\zeta$  are linked by the mapping (1) that transforms streamlines into streamlines and, especially, the unit circle of the  $\zeta$ -plane into  $C$ .

3. To the motion just considered we add a uniform velocity  $U$  directed along the *negative* real axis. We obtain thus a new motion (disturbance of a fluid which is at rest at infinite distance by a cylinder moving through it sidewise to the left). The complex potential of this motion is obviously

$$\chi = \chi' - Uz = U \left[ \lambda \left( \zeta + \frac{1}{\zeta} \right) - z \right] \quad (3)$$

where  $z$  and  $\zeta$  remain linked by (1). (Of course the coördinate system re-

mains fixed with respect to the solid.) The velocity at the point  $z$  is  $\bar{w}$ , conjugate to

$$w = \frac{d\chi}{dz} = U \left[ \lambda \left( 1 - \frac{1}{\zeta^2} \right) \frac{d\zeta}{dz} - 1 \right]. \quad (4)$$

The kinetic energy of a layer of the fluid, of unit thickness and parallel to the  $z = x + iy$  plane, is

$$\frac{1}{2}\rho \iint |w|^2 dx dy = \frac{1}{2} MU^2. \quad (5)$$

The integral is extended over the exterior of  $C$ , and  $M$  is the *virtual mass per unit height*. From (4) and (5) we obtain

$$\begin{aligned} M/\rho &= \iint \left| \lambda \left( 1 - \frac{1}{\zeta^2} \right) \frac{d\zeta}{dz} - 1 \right|^2 dx dy \\ &= \iint \left| \frac{dz}{d\zeta} - \lambda \left( 1 - \frac{1}{\zeta^2} \right) \right|^2 d\xi d\eta; \end{aligned} \quad (6)$$

the latter integral is extended over the exterior of the unit circle in the  $\zeta = \xi + i\eta$  plane. Introducing (1) and polar coördinates, we obtain from (6) in the usual way that

$$M/\rho = \pi\lambda^2(|c_1 - 1|^2 + 2|c_2|^2 + 3|c_3|^2 + \dots). \quad (7)$$

Now the area of  $C$  or, what is numerically the same, the volume  $V$  of the moving cylinder per unit height is

$$V = \pi\lambda^2(1 - |c_1|^2 - 2|c_2|^2 - 3|c_3|^2 - \dots). \quad (8)$$

This is well known and obtained by a computation analogous to the one just sketched. It follows from (7) and (8) that

$$V + M/\rho = 2\pi\lambda^2(1 - \Re c_1). \quad (9)$$

where  $\Re c_1$  denotes the real part of  $c_1$ .

4. Now, we wish to obtain  $M_\alpha$ , the virtual mass per unit height corresponding to a direction of the velocity that includes the angle  $\alpha$  with the direction just considered. We reduce this problem to the foregoing by a rotation, introducing the new complex variables  $z'$  and  $\zeta'$ ,

$$z' = e^{i\alpha} z, \quad \zeta' = e^{i\alpha} \zeta.$$

We obtain from (1) that

$$z' = \lambda \left( \zeta' + c_0 e^{i\alpha} + \frac{c_1 e^{2i\alpha}}{\zeta'} + \frac{c_2 e^{3i\alpha}}{\zeta'^2} + \dots \right) \quad (1')$$

Substituting  $c_1 e^{2i\alpha}$  for  $c_1$  in (9), we obtain

$$V + M_\alpha/\rho = 2\pi\lambda^2(1 - \Re c_1 e^{2i\alpha}) \quad (9')$$

and hence

$$V + M_{\alpha+\pi/2}/\rho = 2\pi\lambda^2(1 + \Re c_1 e^{2i\alpha}) \quad (10)$$

We define  $\bar{M}$ , the average virtual mass per unit height by

$$\bar{M} = (1/2\pi\rho) \int_0^{2\pi} M_\alpha d\alpha = (1/2\rho)(M_\alpha + M_{\alpha+\pi/2}). \quad (11)$$

( $\bar{M}$  has, in fact, the dimension of an area, and so has  $V$ .) From (9'), (10) and (11) we find finally

$$V + \bar{M} = 2\pi\lambda^2 \quad (12)$$

5. Now  $\lambda$  is the so-called outer radius of  $C$  (that is the radius of the circle onto the exterior of which the exterior of  $C$  is so mapped that the points at infinity correspond to each other with unit magnification). It follows from (8) (and is well known) that

$$V < \pi\lambda^2.$$

unless  $C$  is a circle. Therefore, by (12),

$$\bar{M} > V$$

with the same proviso. For the circle, however,  $\bar{M} = V$ . Thus, we have proved *that of all cylinders having the same area of the cross-section, the circular cylinder has the minimum average virtual mass per unit height.*

We can derive another result from (12): *the average virtual mass per unit height decreases by symmetrization.* Indeed, we know that symmetrization leaves  $V$  unchanged and decreases the outer radius  $\lambda$ .<sup>2</sup>

<sup>1</sup> This is suggested by a systematic study of the dependence of the capacity on geometric data which has been undertaken recently by Mr. G. Szegő and the author.

<sup>2</sup> See G. Pólya and G. Szegő, "Inequalities for the Capacity of a Condenser," *Amer. Jour. Math.*, **67**, 1-32 (1945), especially pp. 13-14.





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*A SUPPRESSOR IN NEUROSPORA AND ITS USE AS EVIDENCE  
FOR ALLELISM\**

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In *Drosophila* recessive mutations have been observed which suppress the effect of specific mutations at other loci. Thus an individual, homozygous for a suppressor gene in the mutant form and for the mutant gene upon which the suppressor acts, is phenotypically wild type, or nearly so. Suppressors of black,<sup>1</sup> purple,<sup>2, 3</sup> sable<sup>4</sup> and vermillion<sup>4, 5</sup> are among those which have been reported. In the vermillion case there is evidence<sup>5</sup> which shows that the action of the suppressor is to restore *v*<sup>+</sup> substance (kynurenine), the formation of which is prevented when the mutant gene vermillion is present in the homozygous condition.

A similar situation which has been found in *Neurospora* will be reported here. The effect of a mutation which prevents the synthesis of pyrimidine is partly suppressed by the presence of a mutant gene at a different locus. It seems logical to assume that the presence of the second mutation results in the formation of an intermediate in pyrimidine synthesis which is lacking in the pyrimidineless strain. Evidence that the new mutation is not due simply to duplication of the pyrimidineless locus will be presented.

Results of studies of combinations of the suppressor with five pyrimidineless strains have supplemented other evidence regarding relationships among the mutant genes concerned. Genetic difference is readily demonstrated in *Neurospora*<sup>6</sup> but evidence for allelism is less convincing, because neither the absence of crossing over, nor the heterocaryon test<sup>7</sup> is conclusive. For this reason additional evidence, obtained by use of the suppressor, that three pyrimidineless strains carry allelic genes, is welcome, particularly since these strains show marked physiological differences, and therefore constitute the first series of multiple alleles to be described in *Neurospora*. This series may prove useful, since one would expect biochemical studies to be simpler in *Neurospora* than in more complex

organisms such as corn, *Drosophila* and mammals, in which multiple alleles have been studied in detail.

Forty-five independent occurrences of mutation which results in pyrimidine deficiency have been recorded in *Neurospora*. These were obtained from x-ray and ultra-violet treatment as described by Beadle and Tatum.<sup>8</sup> The deficient strains utilize uracil or the nucleosides and nucleotides of uracil and cytosine, as will be reported in detail elsewhere.<sup>9</sup> One of the five strains to be discussed here, number 263, has been described by Loring and Pierce.<sup>10</sup>

*Crosses to Determine Genetic Relationships.*—Isolation numbers of the strains considered are 263, 38502, 37301, 37815 and 67602, but for convenience they will be referred to here as 1, 2, 3a, 3b and 3c. Each strain behaves in backcrosses as if the difference from the parent wild type were due to modification of one gene. That is, when any one is back-crossed to the parent strain, and the eight ascospores from each of a number of asci are isolated and tested using techniques reviewed by Beadle,<sup>6</sup> four of the eight spores always prove to be like the mutant parent and four like the wild-type parent.

TABLE 1  
CROSSES BETWEEN MUTANT STRAINS

STRAINS CROSSED	NUMBER OF ASCI OBSERVED	ASCI WITH 1 PAIR OF WILD-TYPE SPORES	ASCI WITH 2 PAIRS OF WILD-TYPE SPORES	ASCI WITH 4 PAIRS OF MUTANT SPORES
1 × 3a	37	5	0	32
1 × 3b	94	8	0	86
1 × 2	17	11	0	6
1 × 3c	53	5	0	48
3a × 3b	59	0	0	59
3a × 2	17	10	0	7
3a × 3c	15	0	0	15
3b × 2	19	6	1	12
3b × 3c	100	0	0	100
2 × 3c	21	7	0	14

Table 1 gives results of intercrosses among the five strains. (In all cases here only asci are included from which at least one member of each spore pair germinated.) From these results it appears that 3a, 3b and 3c are alike genetically, while 1 and 2 are different from these three and from each other. This is evident since from crosses among the first three, no asci were observed containing wild-type spores, while all crosses involving 1 and 2 gave some asci of this type. In order to confirm evidence of genetic difference it was demonstrated that an ascus containing wild-type spores also contained spores carrying two mutant genes. This was done by outcrossing a mutant strain derived from such a spore to wild type and recovering asci containing six or eight mutant spores. If an ascus from the

intercross contains four wild-type spores, then obviously the other four spores must carry both mutant genes, but if the ascus contains two wild-type spores then only two of the six mutant spores will give rise to double mutants. If only asci of the latter type are obtained from an intercross it would be necessary to cross to wild type a strain derived from one member of each of the three pairs of mutant spores, were it not often possible to distinguish the double mutants, partly on the basis of the position of spores in the ascus and partly because of slight differences in growth habit. Results from crosses of double mutants to wild type appear in table 2.

It is interesting that the three loci represented are on the same chromosome, as can be seen from the data in tables 1 and 2. The distances from the centromere are, roughly, 12, 39 and 17 units for 1, 2 and 3, respectively.

TABLE 2  
CROSSES BETWEEN DOUBLE MUTANTS AND WILD TYPE

STRAINS CROSSED	NUMBER OF ASCI OBSERVED	ASCI WITH 1 PAIR OF WILD TYPE SPORES	ASCI WITH 2 PAIR OF WILD TYPE SPORES	ASCI WITH 4 PAIRS OF MUTANT SPORES
1, 3a × wild	34	8	26	0
1, 3b × wild	19	3	16	0
1, 2 × wild	12	7	5	0
1, 3c × wild	27	2	25	0
3a, 2 × wild	1	1	0	0
3b, 2 × wild	10	8	1	1
2, 3c × wild	6	4	2	0

*A Suppressor of 3a.*—A strain of 3a, which had begun to grow in the absence of pyrimidine as if a back mutation had occurred, was tested by crossing it to wild type. Two types of asci would be expected if back mutation had taken place, one containing eight wild-type spores, arising from nuclei carrying the back mutation, and the other, from nuclei carrying the mutant gene unchanged, containing four wild-type and four mutant spores. These two types were found, but a third type appeared as well, containing six wild-type and two mutant spores. This third type of ascus cannot be explained on the basis of back mutation at the 3a locus, but, rather, it is necessary to assume that, at a different locus, a mutation had occurred which, in some way, suppresses the effect of the mutation at the 3a locus. If this assumption were correct then all three types of asci would be expected, if there were no close linkage. The second type could result either from nuclei which did not carry the new mutation or from failure of the two mutant genes to combine in any spore pair. The first type could result from combination of the two mutations in four spores, and the third from combination in two spores.

From an ascus with eight wild-type appearing spores one member of each spore pair was crossed to wild type. Two of these crosses gave only

wild-type progeny, while from the other two, the three types of asci just described were recovered, demonstrating that the assumption made above is correct. As a further check, an ascus containing four wild-type and four mutant spores was selected from one of the above two crosses. A wild-type spore from such an ascus should carry the suppressor gene. This proved to be the case, for when a strain from one of these spores was crossed to an unchanged strain of 3a the three ascus types described above were recovered.

TABLE 3  
CROSSES BETWEEN MUTANTS AND THE SUPPRESSOR<sup>a</sup>

STRAINS CROSSED	NUMBER OF ASCI OBSERVED	ASCI WITH 4 PAIRS OF WILD-TYPE SPORES	ASCI WITH 3 PAIRS OF WILD-TYPE SPORES	ASCI WITH 2 PAIRS OF WILD-TYPE SPORES
1 × S	19	0	0	19
3a × S	6	1	1	4
3a-S × wild	38	10	22	6
3b × S	19	2	10	7
2 × S	17	0	0	17
3c × S	11	3	2	6
3c-S × wild	14	4	7	3

<sup>a</sup> In some cases germination of spores from crosses between mutants and the suppressor was very poor. For this reason data from crosses of mutant-suppressor combinations to wild-type are included.

*The Effect of the Suppressor on Other Pyrimidineless Mutants.*—Results, given in table 3, from crosses between the suppressor strain and the remaining four mutants show that the suppressor has no effect when in combination with 1 and 2, since each ascus recovered contained four mutant and four wild-type spores. In order to show that these two mutants had not simply failed to combine with the suppressor each was crossed to a strain carrying both 3a and the suppressor gene in the mutant form. The appearance, among the progeny of both crosses, of asci containing six or eight mutant spores demonstrates that in these asci the two mutants had indeed combined with the suppressor and had not been affected by it. This follows from the fact that in a cross involving the suppressor and two mutants, both of which were capable of being suppressed, no asci could occur containing more than four mutant spores.

The data in table 3 show that 3b and 3c, as well as 3a, are suppressed. This fact is taken as evidence that mutation at the same locus has occurred in the three strains.

*Growth Requirements of the Mutants.*—The three mutants which behave as alleles all have much the same requirement for uridine or hydrolyzed nucleic acid if they are allowed to grow at 35°C., but at 25°C. there are striking differences (Table 4). At this temperature 3a requires nearly the same amount of uridine or hydrolyzed nucleic acid as it does at 35°C.,

while 3c needs only about one sixth as much and 3b grows normally in the absence of any source of pyrimidine. Hence it would appear that the genetic block is complete in the three strains at 35°C., and in 3a at 25°C. also, but is only partial in 3c at 25°C. and seemingly non-existent in 3b at this temperature.

TABLE 4  
GROWTH REQUIREMENT OF MUTANT STRAINS

STRAIN	HYDROLYZED NUCLEIC ACID* REQUIRED FOR 1/2 MAXIMUM GROWTH (MG.)	
	25°C.	35°C.
3a	3.3	3.15
3b	0	2.4
3c	0.38	2.3

\* Ribonucleic acid in 1 N NaOH, 24 hours at 25°C.

Strain 2 has not been thoroughly tested, but the data available indicate that its requirements are similar to those of 3c. Strain 1 has much the same requirement for uridine as 3b, but it differs from the other four strains in that it can utilize orotic acid as a substitute for uridine. Further studies along these lines will be reported elsewhere.<sup>9</sup>

*Growth Characteristics of the Suppressed Mutant.*—Preliminary experiments have shown that growth of a strain carrying the mutant gene 3a and the suppressor is not completely normal in the absence of pyrimidine. The dry weight of mycelium obtained after four days growth at 25°C. in 20 ml. of medium is about 40 mg. while that obtained using a wild-type strain under the same conditions is about 80 mg. Normal growth of the suppressed mutant takes place if any one of the following supplements is added to the medium: 0.5 mg. of uridine, 3 mg. of hydrolyzed nucleic acid, 10 mg. of lysine or 10 mg. of histidine. If the medium contains 0.5 microgram of arginine no growth takes place, although arginine in concentrations as high as 10 mg. in 20 ml. does not affect the growth of the wild type. Citrulline and ornithine are also inhibitory but are far less so than arginine. The quantities of these three compounds which, in 20 ml. of medium will allow half-maximum growth are as follows: arginine 0.15 microgram, citrulline 230 micrograms and ornithine 400 micrograms. Inhibition by arginine can be overcome by hydrolyzed nucleic acid or lysine, but not by histidine. The five mutant strains without the suppressor, when grown on a medium containing a quantity of hydrolyzed nucleic acid which allows half-maximum growth, or less, are not affected by the addition of 5 micrograms of arginine, but show a slight inhibition if 10 mg. of histidine are added.

No interpretation which is consistent with all of these facts has been made as yet, but further experiments are in progress. In connection with the lysine-arginine interaction reported here it should be mentioned that



Doermann<sup>11</sup> has found the lysine-requiring mutants of *Neurospora* to be inhibited if the molar concentration of arginine in the medium exceeds that of lysine.

*Discussion.*—Although it has not been proved directly, there is much evidence which is consistent with the hypothesis that modification of a gene results in a direct change in the activity of one enzyme.<sup>12</sup> On this basis the differences in growth characteristics of the three mutants which behave as alleles would make it appear that different modifications of the same gene are possible, each of which results in a distinct difference in the activity of the enzyme. This view agrees with observations on multiple alleles in other organisms.<sup>13</sup> A study of the properties of the enzyme affected in the three pyrimidineless strains may prove to be useful, but such a study will not be possible until further knowledge of the reaction involved is available.

It would be of interest to know the former condition of the gene which, by mutation, became able to take over the function of the inactive gene at the 3a locus. Perhaps the most obvious possibility is that the wild-type parent carries an inactive duplicate of this locus which was made active by mutation. This possibility would seem to be ruled out, however, by the fact that arginine suppresses the suppressor but does not affect wild type, in which the 3a locus must be active. Also the fact that growth of the mutant strains without the suppressor, on limiting quantities of hydrolyzed nucleic acid, is not affected by arginine would seem to indicate that arginine inhibition of the suppressed mutant is not simply a result of an insufficient supply of pyrimidine.

Two alternative possibilities that have been suggested are, first, that an entirely new gene has arisen from inactive genic material, and, second, that a gene corresponding to an enzyme which promotes a reaction in another synthesis has been modified so that the enzyme now catalyzes the required reaction in pyrimidine synthesis as well. No basis of choice between these two possibilities seems apparent at present.

*Summary.*—A suppressor, comparable to those found in *Drosophila*, has occurred in *Neurospora*. This suppressor acts upon three physiologically different pyrimidine-requiring strains which behave in genetic tests as if they carry allelic genes. It has no effect in combination with two other pyrimidineless strains which are genetically different from these three.

The characteristics of one of the suppressed mutants are sufficiently different from those of wild type to make it unlikely that suppression is due to duplication of the suppressed locus.

\* Work supported by grants from the Rockefeller Foundation.

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*X-RAY AND ULTRAVIOLET STUDIES ON POLLEN TUBE  
CHROMOSOMES. II. THE QUADRIPARTITE STRUCTURE OF  
THE PROPHASE CHROMOSOMES OF TRADESCANTIA\**

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Most x-ray data dealing with induced chromosome breakage are interpretable on the basis of a divided or an undivided chromosome. The extensive studies on induced breaks in the microspore chromosomes of *Tradescantia*<sup>1</sup> indicate that chromatid and chromosome breaks result, respectively, from changes occurring within a divided or an undivided chromosome. Data from pollen tube chromosomes in the same plant,<sup>2</sup> where the split nature of the chromosomes can be determined at the time of treatment, agree with the above view, and, at the same time, invalidate beyond a doubt the interpretation of Darlington and LaCour<sup>3</sup> as to the origin of induced aberrations of the isochromatid type. The relative lack of mosaics in x-rayed *Drosophila*<sup>4</sup> suggests that, for the most part, the chromosomes in the sperm head are undivided at the time of treatment, although chromatid breaks are by no means rare in the treated salivary gland chromosomes. It appears, therefore, that, as a general rule, the smallest subdivision of the chromosome to be involved in breakage and rearrangement is the chromatid. This interpretation explains very well similar breakage results obtained from chromosomes treated with ultraviolet,<sup>5</sup> as well as the extensive data of Stadler<sup>6</sup> on endospermal deficiencies in treated maize.

That the chromatid is not the smallest subdivision of the chromosome, however, has long been known (see Nebel<sup>6, 7</sup> and Kuwada<sup>8</sup> for reviews). In addition to earlier observational studies there is much supporting data

of recent date. Mention may be made of the x-rayed chromosomes of *Tradescantia*<sup>2, 9</sup> and *Steatococcus*,<sup>10</sup> the quadripartite nature of the trabant in the microspore chromosomes of *Tradescantia*,<sup>11</sup> and the occurrence of half-chromatid exchanges in the pollen tube chromosomes of the same plant.<sup>2</sup> Carlson<sup>12</sup> has likewise reported the probable occurrence of half-chromatid breaks in the neuroblast chromosomes of *Chortophaga* embryos, as has Marshak<sup>13</sup> in *Vicia*, but these have been dismissed by some<sup>2</sup> as irrelevant observations. Similar half-chromatid breaks are frequent in the pollen tube chromosomes of *Tradescantia* following x-ray or ultraviolet treatment,<sup>2</sup> although they are admittedly difficult to distinguish from the openings between adjacent somatic coils.

Recently a number of aberrations have been found whose interpretation can only be understood in terms of half-chromatid breaks and rearrangements, and it is the purpose of this note to present a consideration of them. Because of their sporadic appearance, no attempt has been made to relate them to dosage, but they are frequent enough to convince the author that the chromosomes in very early prophase (the time of treatment) are quadripartite.

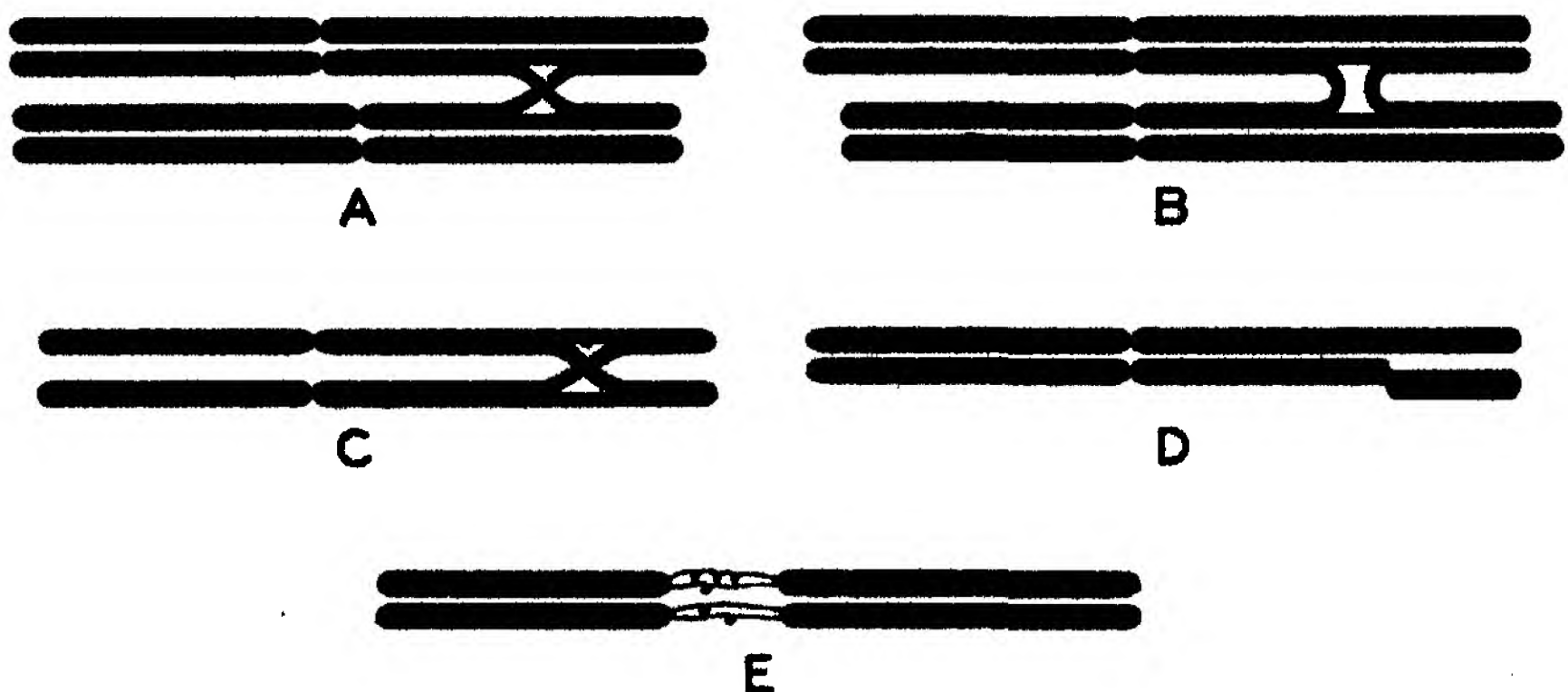


FIGURE 1

Semi-diagrammatic drawings of *Tradescantia* pollen tube chromosomes. A and B. Half-chromatid exchanges between non-homologous chromosomes. C. Half chromatid exchange between the two chromatids of the same chromosome. D. Half-chromatid deletion which has rotated from an inside to an outside position. E. Metaphase chromosome showing a quadripartite condition in the centric region.

Figure 1 illustrates the types of half-chromatid aberrations found. Figures 1A and 1B are exchange breaks. At the points of exchange, the stretched condition of the threads permitted the split in the individual chromatids to be viewed with clarity even though the portions of the chromatids on either side of the break presented a solid and undivided

appearance. Figure 1C illustrates an exchange break between half-chromatids of the same chromosome. The sister chromatids have moved apart from each other at their centromeres, but they are held in close juxtaposition at the point of exchange.

The exchange type of break has never been found following treatment with ultra-violet. The breakage of a single half-chromatid, however, has been observed, and, in figure 1D, the rotation of the broken piece from an inside to an outside position leaves little doubt as to the interpretation: it cannot be confused with the separation of the gyres of a coiled chromatid. Further evidence of the quadripartite structure is illustrated in figure 1E. The chromosomes, in passing down the pollen tube, are frequently stretched, with the strain being evidenced by an attenuation of the centric region. When so stretched, this portion of the chromosome shows four very thin parallel strands, each possessing one or more coils of small diameter. Whether or not these stretched and coiled strands represent the centromere, or a portion of it, is not known; more likely, they are the half-chromatids pulled free from body of the chromatids. If the latter, it may be pointed out that there is no evidence of a chromomeric differentiation even though the threads approximate the thinness of a leptotene chromosome. The coils, too, are of a much smaller diameter than the usual somatic coil.

The fact that the chromatid is further split into half-chromatids in the early prophase stages poses the problem of "What is the functional unit of the chromosome, and what determines its integrity?" In crossing over, genetical and cytological evidence leaves little doubt that the chromatid is that functional unit. The chromatid also appears to be the smallest functional unit in coiling,<sup>13</sup> and, as pointed out above, most x-ray and ultra-violet data are understandable if interpreted in terms of chromatids or chromosomes, but not in terms of some smaller unit. Since, however, the evidence presented reveals a quadripartite structure which under most circumstances behaves as though it were bipartite, the conclusion is unavoidable that there must be some structural unit in the chromosome other than the chromonemata themselves which preserves the integrity of the chromatid, thus enabling it to react as a single entity in crossing over, coiling, breakage, and reattachment. Occasionally this structure can be broken in such a way by radiation as to permit the formation of half-chromatid exchanges, thus revealing a further subdivision of the chromonemata, but under ordinary circumstances the unitary behavior of the chromatid remains. It is tempting to suggest the matrix as the structural unit which determines the behavior of the chromosome, or chromatid, in breakage and reattachment, but the speculative nature of our knowledge concerning the structure and function of the matrix is such as to make one proceed in that direction with caution. It is only possible to state that

the degree to which the chromonemata is subdivided is not the limiting factor which determines the types of breakage and subsequent recombination found following x-radiation. It likewise follows that ultra-violet, although possessing a localized photochemical action, is capable of breaking a bipartite chromatid. Whether it does so by disrupting some structure other than the chromonema itself remains to be determined.

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## ON THE ASYMPTOTIC SOLUTION OF THE DIFFERENTIAL EQUATION FOR SMALL DISTURBANCES IN A LAMINAR FLOW\*

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The hydrodynamical equation of Orr and Sommerfeld (see C. C. Lin<sup>1</sup> for a detailed analysis) is a special case of differential equations of the form

$$\sum_{j=0}^4 a_j y^{(4-j)} + \lambda^2 \sum_{k=0}^2 b_k y^{(2-k)} = 0 \quad (1)$$

Here  $a_j$ ,  $b_k$  are analytic functions of the complex variable  $x$ ,  $\lambda$  is a large complex parameter with constant argument,  $a_0 = 1$ , and  $b_0$  has a zero of first order at some point, say at  $x = 0$ .

The aim of the present work is to supplement the hydrodynamical theory by a mathematical study of the validity of the formal asymptotic developments used there and, in particular, to shed some additional light on the phenomenon of the so-called "crossing substitutions."



For the convenient statement of our results we need a few definitions. We denote by  $Q(x)$  the two-valued function

$$Q(x) = \int_0^x (-b_0(t))^{1/2} dt$$

and by  $\eta(x)$  the function

$$\eta(x) = b_0^{-1/4} \exp \left\{ \frac{1}{2} \int \frac{b_0 a_1 - b_1}{b_0} dt \right\}$$

Let  $S$  be the domain in the  $x$ -plane defined by the inequalities

$$0 < |Q(x)| \leq K,$$

where  $K$  is a constant chosen so that  $S$  does not contain any zeros of  $b_0$ . There exist three curves  $C_1, C_2, C_3$ , along which  $\operatorname{Re}(\lambda Q(x)) = 0$ . These curves meet at the origin and divide  $S$  into three curvilinear sectors  $S_k$ , ( $k = 1, 2, 3$ ). We choose our subscripts so that  $S_k$  is bounded by the two curves  $C_j$ , ( $j \neq k$ ), and we assume that  $C_j$  belongs to  $S_k$ .

We finally introduce a generic symbol  $E(S)$  to denote any function of  $x$  and  $\lambda$  which, together with its first three derivatives, is bounded uniformly for  $|\lambda| \geq R > 0$  and for all  $x$  in any given closed subdomain of  $S$ . The symbols  $E(S_k)$ ,  $E(S - C_k)$ , etc., are similarly defined.

**THEOREM 1.** *There exist solutions  $A_k(x, \lambda)$ , ( $k = 1, 2, 3$ ) of (1) having the asymptotic representations*

$$A_k(x, \lambda) = e^{\lambda Q(x)} \left[ \eta(x) + \frac{E(S - C_k)}{\lambda} \right] \quad (2)$$

Here  $Q(x)$  has to be taken with the determination for which  $\operatorname{Re}(\lambda Q(x)) \leq 0$  in  $S_k$ , and the functions  $Q(x)$  and  $\eta(x)$  are regular analytic in  $S - C_k$ .

*Remark:* Note that  $A_k(x, \lambda)$  is asymptotically equal to two different branches of  $e^{\lambda Q(x)} \eta(x)$  on the two sides of  $C_k$ .

**THEOREM 2.** *Let  $u(x)$  be any solution of the differential equation  $\sum_{k=0}^2 b_k y^{(2-k)} = 0$ . Then there exist solutions  $U_k(x, \lambda)$ , ( $k = 1, 2, 3$ ), of (1) such that*

$$U_k(x, \lambda) = u(x) + \frac{E(S - S_k)}{\lambda^2} \quad (3)$$

Both these theorems can be proved by appropriate modifications of methods used for more general differential equations, but for real  $x$  only, by W. J. Trjitzinsky.<sup>2</sup>

Theorems 1 and 2 combined give us fundamental systems of solutions of (1) with known asymptotic properties in any two out of the three



sectors  $S_1, S_2, S_3$ . The next question is to determine the asymptotic character of the solutions introduced in theorem 2 in the remaining sector  $S_k$ . This question is partially answered in the next two theorems:

THEOREM 3. *There exist solutions  $V(x, \lambda)$  of (1) such that*

$$V(x, \lambda) = v(x) + \frac{E(S)}{\lambda^2} \quad (4)$$

where  $v(x)$  is a solution of  $\sum_{k=0}^2 b_k y^{(2-k)} = 0$  which is regular at  $x = 0$ .

*Remark:* Note that the essential difference between (3) and (4) is that  $E(S)$  is bounded in  $S$  and not only in  $S - S_k$ .

THEOREM 4. *Let  $u(x)$  be a solution of  $\sum_{k=0}^2 b_k y^{(2-k)} = 0$  which is singular at  $x = 0$ , and let  $U_k(x, \lambda)$  be a corresponding solution of (1) such that (3) is true. Then  $U_k(x, \lambda)$  diverges at every interior point of  $S_k$ , as  $\lambda \rightarrow \infty$ .*

The last two theorems can be derived from theorems 1 and 2 by using three sets of fundamental systems, each having a known asymptotic representation in two of the sectors  $S_k$ , and by studying the linear relations between these fundamental systems.

Theorem 4 helps to clarify the questions connected with the so-called "inner friction layers." C. C. Lin and others conclude from the behavior of the solutions which we have called  $A_1, A_2$ , that the particular solutions of (1) occurring in the hydrodynamical application approach a discontinuous behavior at  $C_1$  and  $C_2$ , as  $\lambda \rightarrow \infty$ . Using theorem 4 it is easy to give a complete proof of this statement. Moreover, our results show that  $C_1$  and  $C_2$  are not isolated curves of discontinuity for the limits of these solutions, but that they *diverge in the whole sector  $S_3$* .

It may be remarked, in concluding, that the asymptotic character of  $U_k(x, \lambda)$  can be investigated in greater detail, using somewhat different methods. It turns out that  $U_k(x, \lambda)$  is in  $S_k$  asymptotically equal to a function of the form  $\lambda^{-1/2} e^{\lambda Q(x)} \phi(x)$ , where  $\phi(x)$  is bounded and not identically zero.

\* The results presented in this paper were obtained in the course of research conducted under the sponsorship of the Office of Naval Research.

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# ABSTRACT GROUP GENERATED BY THE QUATERNION UNITS

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According to Alexander Macfarlane's *Ten British Mathematicians*, page 44 (1916), W. R. Hamilton stated in a letter to his son that on October 16, 1843, he cut with a knife on a stone of Brougham Bridge, near Dublin, Ireland, the fundamental formula with the symbols  $i, j, k$ , namely,

$$i^2 = j^2 = k^2 = ijk = -1$$

It is desired to emphasize here the fact that this continued equation is really an equational definition of the abstract non-abelian group of order 8 which is now commonly known as the quaternion group, generated by the quaternion units  $i, j, k$ , and that it seems to be the earliest example of an abstract non-abelian group which had been clearly defined then by a set of generational relations between a set of generating operators of the group.

Such definitions soon thereafter became somewhat popular, especially with American writers on group theory, and their origin has frequently been credited to A. Cayley, who emphasized them somewhat later and embodied their fundamental nature in what is known as Cayley's dictum, "a group is defined by the law of combination of its symbols." What A. Cayley embodied in this dictum had really been involved earlier in what W. R. Hamilton regarded as the discovery of quaternions and had noted by the continued equation stated in the preceding paragraph. Probably no other finite non-abelian abstract group has attracted such wide attention and aroused such great hopes as regards its usefulness as did the quaternion group soon after its discovery by W. R. Hamilton in 1843.

A simple proof of the fact that the noted continued equation is really a usable definition of the quaternion group may be given as follows: It results directly from this equation that the two operators  $i$  and  $j$  are two group operators of order 4 which have a common square and that  $ij = k$  and hence that the transform of  $j$  by  $i$ , that is,  $i^3ji = -ki = -j$ . In other words, the operator  $i$  transforms the operator  $j$  into its inverse. The group of order 4 generated by  $j$  is therefore transformed into itself by the operator  $i$  and involves the square of  $i$ . When the group of order 4 generated by  $j$  is extended by the operator  $i$  there results the well-known group of order 8 which is now commonly called the quaternion group and is characterized by the fact that it contains 6 operators of order 4.

The fact that W. R. Hamilton explicitly laid much stress on the associative law in the combination of operators makes it clearer that he may properly be regarded as the founder of the abstract theory of groups instead

of A. Cayley. On the other hand, W. R. Hamilton did not use the term group but favored the use of his own term quaternions for the development of a theory based upon the special abstract group of order 8 which involves 6 operators of order 4. The associative law was later very commonly embodied in the definitions of an abstract group and the development of these groups did much to emphasize the fundamental importance of this law. These observations in regard to W. R. Hamilton seem to imply that the theory of abstract groups may reasonably be regarded as about ten years older than the assumption that A. Cayley was the founder of this theory would imply. Implicitly, W. R. Hamilton emphasized the importance of the quaternion group in his *Lectures on Quaternions*, page xxvi (1853).

## LIMITS FOR THE NUMBER OF SOLUTIONS OF CERTAIN GENERAL TYPES OF EQUATIONS IN A FINITE FIELD

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Dickson,<sup>1</sup> using cyclotomic integers, obtained an inferior limit for the number of solutions of the congruence

$$x^e + y^e + z^e \equiv 0 \pmod{p} \quad (1)$$

where  $e$  and  $p$  are given primes with  $xyz \not\equiv 0 \pmod{p}$ . Hurwitz<sup>2</sup> gave superior and inferior limits for the number of solutions of the congruence

$$ax^e + by^e + cz^e \equiv 0 \pmod{p}, \quad (2)$$

where  $abcxyz \not\equiv 0 \pmod{p}$ . Recently, the writer has considered generalizations of these results and has arrived at limits both superior and inferior, for the number of solutions of the equation

$$c_1x_1^{a_1} + c_2x_2^{a_2} + \dots + c_sx_s^{a_s} + c_{s+1} = 0 \quad (3)$$

in the  $x$ 's, where the  $a$ 's are integers such that  $0 < a \leq p' - 1$ ;  $s \geq 2$ ; the  $c$ 's belong to a finite field of order  $p'$ ,  $p$  prime, which will be designated by  $F(p')$ ; and

$$c_1c_2\dots c_sx_1x_2\dots x_s \not\equiv 0$$

in  $F(p')$ . As a corollary to this it is possible to show that if we take the  $c$ 's in (3) as rational integers and put  $t = 1$ , so that we have a congruence modulo  $p$  in effect, then for  $p$  sufficiently large the congruence has solutions,

with the  $x$ 's all prime to  $p$ . There is a similar theorem involving algebraic numbers. However, in the present paper we shall confine ourselves to the derivation of certain quadratic relations between the numbers of solutions,  $x^m, y^m$ , of trinomial equations of the type

$$1 + ax^m = by^m \quad (3a)$$

for various values of  $a$  and  $b$  in  $F(p')$ ,  $abxy \neq 0$ , as given in Theorem I. Other quadratic relations connecting such solutions have been given before,<sup>3, 4</sup> but those given here are of a quite different character, and they will be used in another paper in the proofs of the results concerning (3) referred to above.

Consider a finite field of order  $p'$  designated by  $F(p')$ , where  $p$  is a prime. Write  $p' = 1 + mc$ . Let  $g$  be a generator of the cyclic group formed by the non-zero elements of  $F(p')$ . Further let  $(i, j)$  denote the number of solutions  $g^r, g^s$  of

$$1 + g^{i+rm} = g^{j+sm} \quad (3b)$$

if  $r$  and  $s$  are each in the range  $0, 1, \dots, c-1$ , noting that  $g^{mc} = 1$ . If we write ind. for index and  $g^{\text{ind } x} = x$ , represent the index of  $(-1)$ , modulo  $m$ , by  $\epsilon$ , then for any  $i$  and  $j$  it is known<sup>4</sup> that

$$(i, j) = (j + \epsilon, i + \epsilon) = (-j + \epsilon, i - j) = (i - j + \epsilon, -j) = (-i, j - i) = (j - i + \epsilon, -i + \epsilon). \quad (4)$$

Also we have<sup>4</sup>

$$\sum_i (i, 0) = c - 1; \sum_i (i, j) = c, \quad (4a)$$

where  $i \equiv 0, 1, \dots, m-1$ ;  $j = 1, 2, \dots, m-1$  modulo  $m$ . We also note that

$$(i, j) = (i + \alpha m, j + \beta m)$$

for any integers  $\alpha$  and  $\beta$ . A fundamental relation we shall employ in what follows is (Mitchell,<sup>4</sup> his  $\psi$  function defined on p. 165; for  $b = a, a + b = d$ )

$$\psi_{a,d}(\alpha)\psi_{a,d}(\alpha^{-1}) = p' \quad (4b)$$

where

$$\psi_{a,d}(\alpha) = \sum_{i,j} (i, j) \alpha^{-ai+dj}$$

$\alpha$  being a primitive  $m$ th root of unity,  $a$  and  $d$  any integers subject to the conditions  $a \not\equiv 0, d \not\equiv 0, a - d \not\equiv 0 \pmod{m}$  with  $i$  and  $j$  ranging independently over the integers  $0, 1, \dots, m-1$ .

We shall now determine the quantities

$$\sum_{i,j}^{0 \text{ to } m-1} (i, j) (i + h, j + k) = A_{hk} \quad (5)$$

First we determine  $A_{00}$ . Now if  $a \not\equiv 0 \pmod{m}$ ,  $d \not\equiv 0 \pmod{m}$ ,  $d - a \not\equiv 0 \pmod{m}$  then if  $\alpha$  is a primitive  $m$ th root of unity, (4b) gives

$$\sum_{h,j,k}^{0 \text{ to } m-1} (i, j) (i + h, j + k) \alpha^{-ah+dk} = p^t \quad (6)$$

Let  $p^t = 1 + mc$ . Consider the summation

$$\sum_{d=0}^{m-1} \sum_{h,j,k}^{0 \text{ to } m-1} (i, j) (i + h, j + k) \alpha^{-ah+dk} = \sum_{d=0}^{m-1} C_{ad} \quad (7)$$

For  $a \not\equiv 0 \pmod{m}$ ,  $d \not\equiv 0 \pmod{m}$ ,  $a - d \not\equiv 0 \pmod{m}$  each term in the sum with respect to  $d = p^t$ . Now consider the value when  $d = 0$ , which is

$$\sum_{h,j,k}^{0 \text{ to } m-1} (i, j) (i + h, j + k) \alpha^{-ah}$$

Set  $i + h = i'$ ,  $j + k = j'$  then this expression becomes

$$\sum_{i,j,i',j'} (i, j) (i', j') \alpha^{-(i'-i)a} = C_{a0} \quad (8)$$

This is obviously equal to

$$\left( \sum_{i,j}^{0 \text{ to } m-1} (ij) \alpha^{ia} \right) \left( \sum_{i',j'}^{0 \text{ to } m-1} (i'j') \alpha^{-i'a} \right) \quad (8a)$$

and each of these equals  $(-1)$  if  $a \not\equiv 0 \pmod{m}$ , hence

$$C_{a0} = 1 \quad (9)$$

Now consider the case when  $a - d \equiv 0 \pmod{m}$  in (7). The corresponding term reduces to

$$\sum (i, j) (i', j') \alpha^{-a(h-k)} = C_{a0}$$

or

$$\sum (i, j) \alpha^{-a(i-j)} \sum (i', j') \alpha^{-a(i'-j')}$$

If  $i - j = f$ , then for  $a \not\equiv 0 \pmod{m}$ , using (4),

$$\sum_{j,f} (f + \epsilon, -j) \alpha^{-af} = \sum_{j,f} (f, -j - \epsilon) \alpha^{-af} = -1$$

and similarly for the second factor. Hence

$$C_{aa} = 1, a \not\equiv 0 \pmod{m}. \quad (10)$$

Now if  $a \equiv 0 \pmod{m}$  in (8) then we find

$$C_{00} = (mc - 1)^2 \quad (11)$$

employing (8a) and (4a).

We now simplify (7) under the assumption that  $a \not\equiv 0 \pmod{m}$ . Using (7), (8), (9) and (10), we find

$$\sum_{d=0}^{m-1} C_{ad} = (m-2)p' + 2; \quad a \not\equiv 0 \pmod{m}. \quad (12)$$

For the case where  $a \equiv 0 \pmod{m}$  we obtain in the same way that (9) was derived

$$C_{0d} = 1, \quad d \not\equiv 0 \pmod{m}. \quad (13)$$

Hence

$$\sum_{d=0}^{m-1} C_{0d} = (mc - 1)^2 + m - 1 \quad (13a)$$

Now the left hand member of (12) may be written

$$\sum_{d=0}^{m-1} \sum_{hk} A_{hk}(\alpha)^{-ah+dk}$$

where  $A_{hk}$  is defined as in (5).

Now

$$\begin{aligned} & \sum_{d=0}^{m-1} \sum_{hk} A_{hk} \alpha^{-ah+dk} \\ &= \sum_{hk} A_{hk} \alpha^{-ah} (1 + \alpha^k + \dots + \alpha^{(m-1)k}) \\ &= 0 \text{ if } k \not\equiv 0 \pmod{m}. \end{aligned}$$

Hence

$$\sum_{d=0}^{m-1} \sum_{hk} A_{hk} \alpha^{-ah+dk} = mA_{h0} \alpha^{-ah},$$

or from (12),

$$m \sum_h A_{h0} \alpha^{-ah} = (m-2)p' + 2, \quad (14)$$

if  $a \not\equiv 0 \pmod{m}$  and it equals  $(mc - 1)^2 + m - 1$  otherwise.

Hence

$$\sum_{a=0}^{m-1} mA_{h0} \alpha^{-ah} = (m-2)(mc+1)(m-1) + 2(m-1) + (mc-1)^2 + m - 1$$



or

$$m^2 A_{00} = m^2 c^2 + m^3 c - 3m^2 c + m^2$$

or

$$A_{00} = (c - 1)^2 + (m - 1)c \quad (14a)$$

Now for  $a \not\equiv 0 \pmod{m}$  we have from (14)

$$m \sum_h A_{ho} \alpha^{-ah+sa} = \alpha^{sa} ((m - 2)p^t + 2)$$

and for  $a \equiv 0 \pmod{m}$ ,

$$m \sum_h A_{ho} = (mc - 1)^2 + m - 1.$$

Hence

$$m \sum_{a=0}^{m-1} \sum_h A_{ho} \alpha^{-ah+sa} = (mc - 1)^2 + m - 1 - ((m - 2)p^t + 2) = (c^2 - c)m^2,$$

or

$$m^2 A_{ho} = m^2 (c^2 - c),$$

$$A_{ho} = c^2 - c, \quad h \not\equiv 0 \pmod{m}. \quad (15)$$

We shall now show also that

$$A_{ok} = c^2 - c, \quad k \not\equiv 0 \pmod{m}. \quad (16)$$

We have

$$A_{ok} = \sum_{i,j} (i, j)(i, j + k)$$

But by (1)

$$(i, j) = (j + \epsilon, i + \epsilon)$$

$$(i, j + k) = (j + k + \epsilon, i + \epsilon)$$

Hence

$$\sum_{i,j} (i, j)(i, j + k) = \sum_{j+\epsilon, i+\epsilon} ((j + \epsilon) + k, i + \epsilon)(j + \epsilon, i + \epsilon),$$

where  $j + \epsilon$  and  $i + \epsilon$  range over the same set  $0, 1, \dots, m - 1$ , modulo  $m$  as do  $i$  and  $j$  so that this equation gives (16), using (15).

We lastly determine  $A_{hk}$  with  $h \not\equiv 0, k \not\equiv 0 \pmod{m}$ . Consider the sum

$$\sum_{d=0}^{m-1} \sum_{h,j,k}^{0 \text{ to } m-1} (ij)(i + hj, j + k) \alpha^{-ah+dk-nd} = B_{ad} \quad (17)$$

We know from (6) that each term corresponding to a given  $d$  equals  $p^t \alpha^{-nd}$  except when  $d \equiv 0, a \equiv 0$ , or  $d \equiv a_1 \pmod{m}$ . For  $d \equiv 0$ , then

the corresponding term equals unity if  $a \not\equiv 0 \pmod{m}$ , using (9). For  $d \equiv a$  we have by (10) that the term is  $\alpha^{-av}$ . Hence

$$B_{adv} = 1 + \alpha^{-v} p^t + \alpha^{-2v} p^t + \dots + \alpha^{-(m-1)v} p^t + \alpha^{-av} - \alpha^{-av} p^t$$

$$B_{adv} = 1 - p^t + \alpha^{-av}(1 - p^t) \quad (18)$$

But  $B_{adv}$  can also be written as

$$mA_{hv}\alpha^{-ah} + \sum_{\substack{k=0 \\ k \neq v}}^{m-1} A_{hk}\alpha^{-ah}(1 + \alpha^{k-v} + \dots + \alpha^{(m-1)(k-v)})$$

and the last summation is 0. Hence by (18)

$$mA_{hv}\alpha^{-ah} = 1 - p^t + \alpha^{-av}(1 - p^t)$$

or

$$A_{hv}\alpha^{-ah} = -c - \alpha^{-av}c \quad (19)$$

and

$$A_{hv}\alpha^{-ah+sa} = -c\alpha^{sa} - c\alpha^{-av+sa} \quad (20)$$

We also have, from (17), for  $a \equiv 0 \pmod{m}$

$$B_{adv} = mc^2 - 2c \quad (21)$$

for if we take (17) with  $a \equiv 0 \pmod{m}$  then for  $d \equiv 0 \pmod{m}$ , we find that the corresponding term is  $(mc - 1)^2$  while the other terms, corresponding to each  $d$  are  $\alpha^{-v}$ ,  $\alpha^{-2v}$ ,  $\dots$ ,  $\alpha^{-(m-1)v}$ , using (13). From (20) and (21) we find

$$\sum_{a=0}^{m-1} A_{hv}\alpha^{-ah+sa} = \sum_{a=0}^{m-1} (-c\alpha^{av} - c\alpha^{-av+sa}) + mc^2 - 2c = mc^2,$$

if  $v \not\equiv s \pmod{m}$ , or

$$A_{sv} = c^2; \quad s \not\equiv 0, \quad v \not\equiv 0, \quad s \not\equiv v \pmod{m}. \quad (21a)$$

For  $v = s$  we have from the above

$$A_{ss} = c^2 - c; \quad s \not\equiv 0 \pmod{m}. \quad (22)$$

Hence we have, employing (14a), (15), (16), (21a) and (22),

**THEOREM I.** Set

$$A_{hk} = \sum_{i,j}^{0 \text{ to } m-1} (i, j)(i + h, j + k),$$

where  $(i, j)$  is the number of sets of values  $f$  and  $l$  in the set  $0, 1, \dots, c - 1$ , which satisfy the equation

$$1 + g^{i+fm} = g^{+m}$$

in the finite field  $F(p')$  where  $p$  is a prime such that  $p' = 1 + cm$ ,  $g$  being a primitive root in  $F(p')$ . Then

$$A_{oo} = (c - 1)^2 + c(m - 1); \quad (23)$$

$$A_{ho} = A_{ok} = c^2 - c, \quad h \not\equiv 0, \quad k \not\equiv 0 \pmod{m}; \quad (24)$$

$$A_{hk} = c^2, \quad \text{with } h \not\equiv k \pmod{m}; \quad h \not\equiv 0, \quad k \not\equiv 0 \pmod{m}; \quad (25)$$

$$A_{hk} = c^2 - c, \quad (26)$$

if  $h \equiv k \pmod{m}$ .

<sup>1</sup> *Crelle*, **135**, 181-188 (1909). Cf. also Pellet, *Bull. Math. Soc. France*, **15**, 80-93 (1888).

<sup>2</sup> *Crelle*, **136**, 272-292 (1909).

<sup>3</sup> THESE PROCEEDINGS, **32**, 47-52 (1946).

<sup>4</sup> Mitchell, *Proc. Amer. Math. Soc.*, **17**, 167 (1916).

## CHARACTERISTIC COÖRDINATES FOR HYPERBOLIC DIFFERENTIAL EQUATIONS IN THE LARGE

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Partial differential equations of hyperbolic type are habitually treated by the introduction of *characteristic coördinates*. When such equations occur in physical problems their solutions in the large are necessarily demanded. In spite of this fact the existence of characteristic coördinates appears to be established only locally. We shall here give conditions for the existence of a (1, 1) differentiable transformation defining the characteristic coördinate system *in the large*. The form of these conditions is suitable for practical application but such applications of the theorem will not be included in this communication.

Let  $D$  be an open simply connected two dimensional domain, finite or infinite, referred to a system of rectangular coördinates  $x^\alpha$ . At each point of  $D$  characteristic directions  $\lambda$  are defined as the solutions of the equation  $\Sigma g_{\alpha\beta} \lambda^\alpha \lambda^\beta = 0$  in which the summation is over the values 1, 2 of the indices and it is supposed that the coefficients  $g_{\alpha\beta}$  are continuous and have continuous first partial derivatives in  $D$ . Assuming  $\det. |g_{\alpha\beta}| < 0$  (hyperbolic case) there will be exactly two distinct characteristic directions at each point of  $D$  and these directions will generate two families or congruences of characteristic curves each of which will cover  $D$  completely. In fact any point  $P$  of  $D$  is contained in a local coördinate system, e.g., a

system obtained by rotation of the  $x$  coördinate neighborhood of  $P$ , within which  $g_{22} \neq 0$  and relative to this system the characteristic curves of either congruence are given as solutions of a differential equation of the form  $dy/dx = f(x, y)$  where the function  $f(x, y)$  is continuous and has continuous partial derivatives with respect to  $x$  and  $y$ . Each characteristic curve therefore has a parametric representation  $x^a(s)$ , admitting continuous first derivatives with respect to the parameter  $s$  which can be taken as the arc length, and such that  $(dx^1/ds)^2 + (dx^2/ds)^2 \neq 0$  at any point of the curve. For brevity such a curve is said to be *regular*. Evidently a characteristic curve cannot end at a point of  $D$  (interior point); hence a characteristic curve can be continued indefinitely in either direction through a point of  $D$  or it can be continued until it meets a boundary point of  $D$  at which point the curve will be considered to terminate.

Now we consider a regular curve  $C$  in  $D$  defined parametrically by  $x^a(p)$  for  $a < p < b$  where either  $a$  or  $b$  can be finite or infinite. The curve  $C$  will be said to be a *cross section* for a congruence of curves covering  $D$  under the following conditions: (1) each curve of the congruence meets  $C$  in one and only one point and (2) a curve of the congruence is not tangent to  $C$  at its point of intersection with  $C$ . We now make the following fundamental assumption. *Each of the above congruences of characteristic curves in  $D$  admits a cross section.*

Denoting one of the characteristic congruences by  $A$  and the other by  $B$ , let  $C_A$  and  $C_B$  be the cross sections of  $A$  and  $B$ . Let  $C_A$  and  $C_B$  have the parametric representations  $x^a(p)$  with  $a < p < b$  and  $x^a(q)$  with  $c < q < d$ , respectively. Let  $h(p)$ ,  $a \leq p \leq b$ , be a monotonically increasing (or decreasing) and continuously differentiable function of  $p$  and  $k(q)$ ,  $c \leq q \leq d$ , an analogous function of the variable  $q$ . Now define the function  $\phi(x)$  over  $D$  by the condition that  $\phi(x)$  be constant along the individual curves of the congruence  $A$  and that it assumes a value on any particular curve of  $A$  equal to the value of the function  $h(p)$  for the value of the parameter  $p$  at the point where the curve cuts  $C_A$ . In a corresponding way we define a function  $\psi(x)$  over  $D$  using the characteristic congruence  $B$ , the cross section  $C_B$  and the function  $k(q)$ . It is evident that both functions  $\phi(x)$  and  $\psi(x)$  are continuous and bounded over  $D$ .

We now prove that the functions  $\phi(x)$  and  $\psi(x)$  have continuous first partial derivatives in  $D$ . Neighboring any point  $Q$  of  $D$  we can make a continuous differentiable transformation,  $x \longleftrightarrow y$ , to a  $y$  coördinate system relative to which the  $A$  and  $B$  curves are the parametric lines  $y^1$  and  $y^2$ , respectively (local characteristic coördinate theorem). Hence in the  $y$  system  $\phi = \phi(y^2)$  and  $\psi = \psi(y^1)$ . Two such systems  $y$  and  $\bar{y}$  are evidently related by a transformation of the form  $y^1 = f(\bar{y}^1)$ ,  $y^2 = g(\bar{y}^2)$  in their common domain where the functions  $f$  and  $g$  have continuous non-vanishing derivatives. Now let  $Q$  be any point in  $D$  and consider the  $A$  curve

through  $Q$  which intersects  $C_A$ . Denote this point of intersection by  $P$ . The arc  $PQ$  of the  $A$  curve can be covered by a finite number  $m$  of the above local coördinate systems. Denote these systems by  $y_1, \dots, y_m$  and suppose that system  $y_1$  contains the point  $P$ , system  $y_m$  the point  $Q$ , and furthermore that any two successive systems  $y_i, y_{i+1}$  contain a region of intersection so that it is possible to traverse the arc  $PQ$  by passing through these local systems in the order  $y_1, \dots, y_m$ . Since  $\phi = \phi(y_m^2)$  in the  $y_m$  system the derivative  $\partial\phi/\partial y_m^1$  exists at  $Q$  and is equal to zero. It suffices therefore to prove the existence and continuity of the derivative  $d\phi/dy_m^2$  at  $Q$ . For this purpose we write

$$\frac{\Delta\phi}{\Delta y_m^2} = \frac{\Delta h}{\Delta p} \frac{\Delta p}{\Delta y_1^2} \frac{\Delta y_1^2}{\Delta y_2^2} \cdots \frac{\Delta y_{m-1}^2}{\Delta y_m^2}. \quad (1)$$

The last factor in the right member of (1) can be considered as the ratio of the increments  $\Delta y_{m-1}^2$  and  $\Delta y_m^2$  at a point on the arc  $PQ$  in the intersection of the  $y_{m-1}$  and  $y_m$  coördinate regions. A similar remark applies to preceding ratios up to and including the ratio  $\Delta y_1^2/\Delta y_2^2$  where the increments are taken at a point on the arc  $PQ$  in the intersection of the  $y_1$  and  $y_2$  regions. These ratios approach limits since the local coördinate transformations involved are differentiable. In the  $y_1$  system the curve  $C_A$  is represented by  $y_1^a(p)$ . Hence  $dy_1^2/dp$  exists and this derivative is different from zero since the curves  $y_1^2 = \text{const.}$  are not tangent to  $C_A$ . Hence the inverse relation  $p = p(y_1^2)$  exists and is differentiable. This proves the existence of the limit of  $\Delta p/\Delta y_1^2$ . Finally the limit  $\Delta h/\Delta p$  exists since the function  $h(p)$  is differentiable by hypothesis. We thus deduce the existence of the derivative  $d\phi/dy_m^2$  by taking the limit of the right member of (1) as  $\Delta y_m^2 \rightarrow 0$ ; moreover, this derivative is seen to be continuous since it is expressible as the product of derivatives each of which is continuous. The existence and continuity of the derivatives of the function  $\phi(x)$  with respect to the original variables  $x^a$  follows from the fact that the local transformation  $x \leftrightarrow y_m$  possesses continuous partial derivatives. In a similar manner we prove the existence and continuity of the partial derivatives of the function  $\psi(x)$ .

The derivative  $d\phi/dy_m^2$  is not equal to zero at  $Q$  since each of the derivatives which results by taking the limit of the right member of (1) is observed to be different from zero. Similarly  $d\psi/dy_m^1$  is different from zero at  $Q$ . Hence, the functional determinant of  $\phi, \psi$  relative to the local  $y$  system is not equal to zero. From this it follows that the functional determinant relative to the original coördinates  $x^a$  is not equal to zero at any point  $Q$  of  $D$  since this determinant is a scalar density under coördinate transformations (the functions  $\phi$  and  $\psi$  being absolute scalars). The transformation  $\phi = \phi(x), \psi = \psi(x)$  is therefore (1, 1) locally.

But the above transformation  $\phi(x), \psi(x)$  is easily seen to be (1, 1) in the large, i.e., over the entire domain  $D$ . For suppose two distinct points  $P$  and  $Q$  of  $D$  are transformed into the same point  $(\phi, \psi)$ . Then  $P$  and  $Q$  must lie on the same  $A$  curve and also on the same  $B$  curve. Introducing the above mentioned parameter  $s$  for the  $A$  curve we see that for some point  $M$  on this curve between  $P$  and  $Q$  we must have

$$\sum \frac{\partial \phi}{\partial x^\alpha} \frac{dx^\alpha}{ds} = 0, \quad \sum \frac{\partial \psi}{\partial x^\alpha} \frac{dx^\alpha}{ds} = 0;$$

the first of these equations follows since  $\phi = \text{const.}$  along the  $A$  curve and the second from the mean value theorem and the assumption that the function  $\psi$  has the same value at points  $P$  and  $Q$ . Since not both derivatives  $dx^1/ds$  and  $dx^2/ds$  vanish simultaneously the determinant of the coefficients  $\partial \phi / \partial x^\alpha$  and  $\partial \psi / \partial x^\alpha$  in the above equations must vanish at the point  $M$ . But this contradicts the fact, already proved, that this determinant is nowhere zero in  $D$ . This proves the above italicized statement.

Now  $\sum (\partial \phi / \partial x^\alpha) \lambda^\alpha = 0$  along any  $A$  curve. Hence the vector having components  $\partial \phi / \partial x^\alpha$  is perpendicular to the vector  $\lambda$  which generates the  $A$  curves. Putting  $\epsilon_{\alpha\beta} = \sqrt{-g} e_{\alpha\beta}$ , where  $e_{11} = e_{22} = 0$ ,  $e_{12} = -e_{21} = 1$  and  $g = \det. |g_{\alpha\beta}|$ , we can therefore write  $\partial \phi / \partial x^\alpha = \gamma \epsilon_{\alpha\mu} \lambda^\mu$  where  $\gamma$  is a proportionality factor. Hence

$$\sum g^{\alpha\beta} \frac{\partial \phi}{\partial x^\alpha} \frac{\partial \phi}{\partial x^\beta} = \gamma^2 \sum g^{\alpha\beta} \epsilon_{\alpha\mu} \epsilon_{\beta\nu} \lambda^\mu \lambda^\nu = \gamma^2 \sum g_{\mu\nu} \lambda^\mu \lambda^\nu = 0.$$

There is a similar equation for the function  $\psi(x)$ . It follows that  $g^{11} = g^{22} = 0$  relative to the  $(\phi, \psi)$  coordinate system. This condition can also be expressed by writing  $g_{11} = g_{22} = 0$ . The components  $g^{12}$  and  $g_{12}$  must be everywhere different from zero relative to the  $(\phi, \psi)$  system since  $\det. |g_{\alpha\beta}| < 0$  over  $D$ .

The results obtained can be summarized by the following theorem. *Let  $D$  be an open simply connected domain, finite or infinite, which is referred to a system of rectangular coordinates  $x^\alpha$ . Suppose that each of the two characteristic congruences defined over  $D$  by the equation  $\sum g_{\alpha\beta} \lambda^\alpha \lambda^\beta = 0$  admits a cross section, where  $\det. |g_{\alpha\beta}| < 0$  and the coefficients  $g_{\alpha\beta}$  are continuous and have continuous first partial derivatives in  $D$ . Then there exist functions  $\phi(x)$  and  $\psi(x)$  which are continuous and have continuous first partial derivatives over  $D$  such that (i) these functions define a (1,1) map of  $D$  into a bounded region of the two dimensional number space of coordinates  $\phi, \psi$  and (ii) the components  $g_{11} = g_{22} = 0$  when taken relative to the  $(\phi, \psi)$  system. The coordinates  $\phi, \psi$  thus defined are called characteristic coordinates. Actually our demonstration of this result has not made explicit use of the condition that  $D$  be simply connected. However it is evident that the region covered by the  $\phi, \psi$  coordinate system is simply connected; from*



this fact and the continuity of the map we infer the simple connectivity of the domain  $D$ .

*Remark 1.* In certain cases the conditions of the above theorem will not apply to a particular domain  $D$  but will apply if the domain is suitably extended. For example consider the infinite strip  $D$  between the lines  $x = \pm 1$ . If one of the congruences consists of the segments of parabolas  $y = (x - a)^2$  contained in  $D$ , where  $a$  is an arbitrary parameter, the  $y$  axis can be taken as the cross section. However if  $D$  is limited to the interior of the square  $x = \pm 1, y = \pm 1$  or even to the infinite region between the lines  $x = \pm 1$  and above the line  $y = 1$ , it is evident that no cross section is possible without the identification of certain segments of parabolas. In practical applications, however, it is expected that one will be in a position to choose the appropriate domain  $D$  for which the simplified conditions of the theorem, as stated, will apply.

*Remark 2.* If we do not impose the condition that the congruences of characteristic curves admit cross sections it can be proved that in any simply connected region  $R$  whose closure  $\bar{R}$  is contained in  $D$  functions  $\phi(x)$  and  $\psi(x)$  can be defined which (1) are continuous and have continuous first partial derivatives in  $R$  and (2) define a locally  $(1, 1)$  transformation to characteristic coördinates. But now the transformation is not necessarily  $(1, 1)$  over the entire region  $R$ .

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## PHYSICAL CURVES

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1. We consider the motion of a particle in the plane under the action of any positional field of force. The general equations of motion are

$$\frac{d^2x}{dt^2} = \phi(x, y), \quad \frac{d^2y}{dt^2} = \psi(x, y), \quad (1)$$

where  $(\phi, \psi)$  are the rectangular components of the force acting at any point  $(x, y)$ , and the mass of the particle is assumed to be unity.

The differential equation of third order representing the system of trajectories, found by eliminating the time  $t$  from (1), is

$$(\psi - y'\phi)y''' = [\psi_x + (\psi_y - \phi_x)y' - \phi_y y'^2]y'' - 3\phi y''^2. \quad (2)$$

This is not an arbitrary differential equation of third order. Hence the

system of  $\infty^3$  trajectories generated by a field of force must have peculiar geometric properties.

I have developed the differential geometric aspects of dynamics in the Princeton Colloquium.<sup>1</sup> A completely characteristic set of five properties of the system of  $\infty^3$  trajectories defined by the differential equation (2) was discussed and I obtained also many other equivalent characteristic sets.<sup>2</sup>

Of importance in dynamics are the  $\infty^1$  lines of force. These are defined by the differential equation of first order

$$dy/dx = y' = \psi/\phi \quad (3)$$

This family has no peculiar geometric properties since any family of  $\infty^1$  curves can represent the lines of force of some positional field.

2. In connection with a field of force, the only curves usually studied are the lines of force and the trajectories. However, other noteworthy systems of curves are connected with the field, for example, brachistochrones, catenaries, velocity curves.

*All these systems, together with the trajectories, may be obtained as special cases of one simple general physical problem:* to find curves along which a constrained motion is possible such that the pressure is proportional to the normal component of the force.

3. If an arbitrary curve is drawn in the plane field of force, and the particle (of unit mass) is started along it from one of its points with a given speed, the constrained motion along the given curve is determined. The acceleration along the curve is given by  $T$ , the tangential component of the force vector. So the speed at any point is determined by

$$v^2 = 2 \int T ds. \quad (4)$$

The pressure  $P$  (of course, normal to the curve since the curve is considered smooth) is given by the elementary formula

$$P = \frac{v^2}{r} - N, \quad (5)$$

where  $N$  is the normal component of the force vector and  $r$  is the radius of curvature of the curve. If we increase the initial speed, the effect is to increase  $v^2$  by a constant  $c$ ; and hence  $P$  changes by the addition of a term of the form  $c/r$ .

4. If the given curve is a trajectory, the initial speed may be so chosen that the pressure vanishes throughout the motion; that is, trajectories may be defined as curves of no constraint. Of course, if a different initial speed is used,  $P$  will be of the form  $c/r$ ; but as regards the curves, they are completely characterized by  $P = 0$ .

If the given curve is a brachistochrone and if the motion along it is brachistochronous (minimum time), Euler proved (assuming that the force

is conservative) that the pressure is double the normal component of the acting force and opposite to it in direction, that is,  $P = -2N$ . If the force is not conservative, the real brachistochrones, as defined by a problem of the calculus of variations, forms a quadruply infinite system. The curves defined by the property  $P = -2N$  then form a triply infinite system of what should be called pseudo-brachistochrones. These curves are actually brachistochrones only in the conservative case. No ambiguity, however, will arise by terming the system here considered brachistochrones instead of pseudo-brachistochrones.

5. The general physical problem suggested is to find curves such that  $P$  shall be proportional to  $N$ , that is,  $P = kN$ . To a given value of  $k$ , there correspond  $\infty^3$  such curves: the system so obtained will be denoted by  $S_k$ .

The four cases of main physical interest are as follows:

$k = 0$  gives  $S_0$ , the system of trajectories;

$k = -2$  gives  $S_{-2}$ , the system of brachistochrones;

$k = 1$  gives  $S_1$ , the system of catenaries;

$k = \infty$  gives  $S_\infty$ , the system of velocity curves.

The last case requires a justification in terms of limits which is easily carried out analytically.

The third case follows from the known fact that when an inextensible flexible homogeneous string is suspended in any field of force, the resulting form of equilibrium, called a catenary in the general sense of the term, has the dynamical property that, when a particle, started out with the proper initial velocity, rolls along the curve, the pressure at any point equals the normal component of the force; that is, catenaries are defined by  $P = N$  corresponding to  $k = 1$ .

6. Of course a triply infinite system  $S_k$  exists for any value of the parameter  $k$ . The differential equation of the system, in intrinsic form, is obtained by eliminating  $v$  from the equations

$$v^2/r = (k + 1)N, \quad vv_s = T. \quad (6)$$

The result is

$$Nr_s = nT - rN_s, \quad (7)$$

where  $n = 2/(k + 1)$ .

The differential equation of third order defining the system  $S_k$  of  $\infty^3$  curves is explicitly

$$(\psi - y'\phi)y''' = [\psi_x + (\psi_y - \phi_x)y' - \phi_y y'^2]y'' - \left[ 3\phi + \frac{(n-2)(\phi + y'\psi)}{1 + y'^2} \right] y''^2. \quad (8)$$

This obviously reduces to the familiar trajectory equation (2) when  $n = 2$

corresponding to  $k = 0$ . Brachistochrones correspond to  $n = -2$ , catenaries to  $n = 1$ , velocity curves to  $n = 0$ . From (8) we derive the fundamental theorem of section 9.

7. Now we state the characteristic properties of a system  $S_k$  of the above type for any value of  $n$ , that is, any value of  $k$ .

*Property 1.* For any given lineal element  $(x, y, y')$ , the foci of the osculating parabolas of the single infinity of curves determined by the given element lie on a circle passing through the given point.<sup>3</sup>

*Property 2.* At any point 0, the tangent of the angle which the focal circle makes with the given element is to the tangent of the angle which the given element makes with a certain fixed direction at 0 (the direction of the acting force) as 3 is to  $n + 1$ , that is, as  $3k + 3$  is to  $k + 3$ .

*Property 3.* Through a given point, there pass a single infinity of curves admitting hyperosculating circles of curvature; the centers of these circles lie on a conic passing through the given point in the direction of the force vector.

*Property 4.* The normal at the given point 0 cuts the conic described in Property 3, at a distance equal to  $n + 1$ , that is  $(k + 3)/(k + 1)$ , times the radius of curvature of the line of force passing through 0.

*Property 5.* This is of the same form as Property V obtained in the discussion of trajectories, the number 3 being replaced by the number  $n + 1$ . When the point 0 is moved, the associated conic referred to above changes in the following manner. Take any two fixed perpendicular directions for the  $x$  direction and the  $y$  direction; through 0 draw lines in these directions meeting the conic again at  $A$  and  $B$ , respectively. Also construct the normal at 0 meeting the conic again at  $N$ . At  $A$  draw a line in the  $y$  direction meeting this normal in some point  $A'$  and at  $B$  draw a line in the  $x$  direction meeting the normal in some point  $B'$ . The variation property referred to takes the form (where  $\omega = \psi/\phi = \text{slope of force vector}$ ).

$$\frac{\partial}{\partial x} \frac{1}{AA'} + \frac{\partial}{\partial y} \frac{1}{BB'} + \frac{\omega\omega_{xy} - \omega_x\omega_y}{(n + 1)\omega^2} = 0. \quad (9)$$

See the diagram on page 11 of the Princeton Colloquium.

8. While the properties corresponding to different values of  $k$  are analogous, they are, of course, not identical. The first property is common to all the systems. But the second property involves the parameter  $k$ . Thus while for trajectories the constant ratio that appears is 1 (bisection), it is  $-3$  for brachistochrones,  $3/2$  for catenaries, and 3 for velocity curves. Not only are the triply infinite systems  $S_k$ , corresponding to different values of  $k$ , distinct in any given field of force, but also no two systems arising in two distinct fields can ever coincide. For example, if a certain system of  $\infty^3$  curves arises as trajectories in one field, it cannot also arise as catenaries in either the same or another field.

9. FUNDAMENTAL THEOREM. *It is found that if a curve of the system  $S_k$  starts in the direction of the line of force, the curvature of this curve is, in general, zero. But for one special curve, it is found that the ratio  $\rho$  of the curvature of the curve to the curvature of the line of force is<sup>4</sup>*

$$\rho = \frac{1}{n+1} = \frac{k+1}{k+3}. \quad (10)$$

For trajectories the special curve is obtained by starting the particle from rest, and the ratio is  $\rho = 1/3$  as in our original theory. For brachistochrones  $\rho = -1$ , for catenaries  $\rho = 1/2$ , for velocity curves  $\rho = 1$ .

10. If we combine all the systems  $S_k$  in a given field of force, we obtain a quadruply infinite system which we now proceed to study. The differential equation of the fourth order defining this system is obtained by eliminating  $k$  from the equation of  $S_k$ . We shall not write this differential equation of fourth order but state some of the geometric properties.

For any given curvature element  $(x, y, y', y'')$ , the  $\infty^1$  curves of the system have the property that the locus of the third center of curvature is a parabola with axis parallel to the fixed radius of curvature, that is, perpendicular to the initial direction  $y'$ . However, the best statement is: *If for each of the curves, we construct the osculating conic (five-point contact), the locus of the centers of these conics is a conic passing through a given point in the given direction.*<sup>5</sup>

11. Appell showed that any collineation carries the  $\infty^3$  dynamical trajectories of a field of force into the trajectories of a new field of force. We have shown that the collineation group is the largest group of transformations with this property, not only for point transformations, but also for contact transformations.

Goursat proved that a conformality converts the dynamical trajectories of a conservative field of force into the trajectories of a conservative field. Our final result is that the conformal group of transformations consists of all the correspondences that transforms the trajectories of a conservative field of force into the trajectories of a conservative field. This applies not only to the  $\infty^2$  curves for a given energy constant (natural family), but also to the total system of  $\infty^3$  trajectories.

The extension of the transformation theories of Appell and Goursat to catenaries and all systems  $S_k$  will be studied elsewhere.

<sup>4</sup> Kasner, Edward, "Differential-Geometric Aspects of Dynamics," Amer. Math. Soc. Colloquium Publications, vol. 3, 1913, 1934 (referred to as Princeton Colloquium). Also see a series of papers in *Trans. Amer. Math. Soc.*, vols. 7-11 (1906-1910). Tautochrones (isochronous motion) are not included among systems  $S_k$  of the present paper.

<sup>5</sup> Recently Terracini has given an alternate projective characterization of the  $\infty^3$  dynamical trajectories. See "sobre la ecuacion diferencial  $y'''' = G(x, y, y')y'' + H(x, y, y')y'^3$ ," *Revista de Matematicas*, 2, 245-329 (1941), Tucuman, Argentina.

<sup>3</sup> Kasner, Edward, and De Cicco, John, "A Generalized Theory of Dynamical Trajectories," *Trans. Amer. Math. Soc.*, **54**, 23-38 (1943).

<sup>4</sup> Kasner, Edward, and Mittleman, D., "A General Theorem on the Initial Curvature of Dynamical Trajectories," *Proc. Nat. Acad. Sci.*, **28**, 48-52 (1942). Also "Extended Theorems in Dynamics," *Science*, **95**, 249-250 (1942). Also a long paper appearing in the current volume of *Revista de Matematicas*.

<sup>5</sup> Kasner, Edward, "Systems of Extremals in the Calculus of Variations," *Bull. Amer. Math. Soc.*, **13**, 290 (1907).





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## REACTIVATION OF IRRADIATED BACTERIOPHAGE BY TRANSFER OF SELF-REPRODUCING UNITS

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In investigating certain peculiarities in the titration of the activity of bacteriophages after ultraviolet irradiation, a mechanism was discovered that offers direct support for interpreting inactivation of these viruses by radiation as due to lethal mutations. Moreover, this mechanism reveals unexpected features of the process of virus reproduction. We summarize here the results of this work and discuss some of their implications. They will be published in detail elsewhere.

Our work employed the coli bacteriophages, T1-T7, their "r" mutants (i.e., with rapid lysis, and large plaque), the host strain, *Escherichia coli* B, and a number of bacterial mutants resistant to one or another of the bacteriophages.<sup>1, 2</sup>

The rate of inactivation of these bacteriophages exposed to ultraviolet light (wave-length 2537 Å) in a non-absorbent medium is a simple logarithmic function of the dose of irradiation (Fig. 1).<sup>3, 4</sup> This indicates a "one hit" mechanism of inactivation, one quantum being the effective inactivating hit. The phage survival is generally measured by plaque count, by diluting the irradiated phage rather heavily in broth and plating the diluted samples with sensitive bacteria. Each phage particle that remains active will give one plaque (phage colony).

It was, however, noticed by Delbrück and Bailey (personal communication) that the active titre of an irradiated suspension, as determined by plaque count, is dependent on the concentration of the irradiated sample when first placed in contact with the bacterial cells. For example, an irradiated sample can be diluted 1:10 into a heavy bacterial suspension, and after a few minutes—during which no phage liberation occurs—diluted again 1:10<sup>4</sup> in broth and plated. The count will be much higher than if the same sample had first been diluted 1:10<sup>4</sup> in broth, then 1:10 in bacterial suspension. This phenomenon seemed to indicate the presence in irradi-

ated phage suspensions of some "factor," besides the phage itself, capable of causing reactivation of inactivated phage particles if allowed to act on the same bacterial cells. A study of this factor was the original purpose of the work reported here.

It has been shown previously,<sup>1</sup> and again in the experiments discussed in this paper, that ultra-violet inactivated phage particles are still adsorbed by sensitive bacteria at the same rate as active particles, even after having received as many as 40-50 lethal hits. This can be demonstrated because

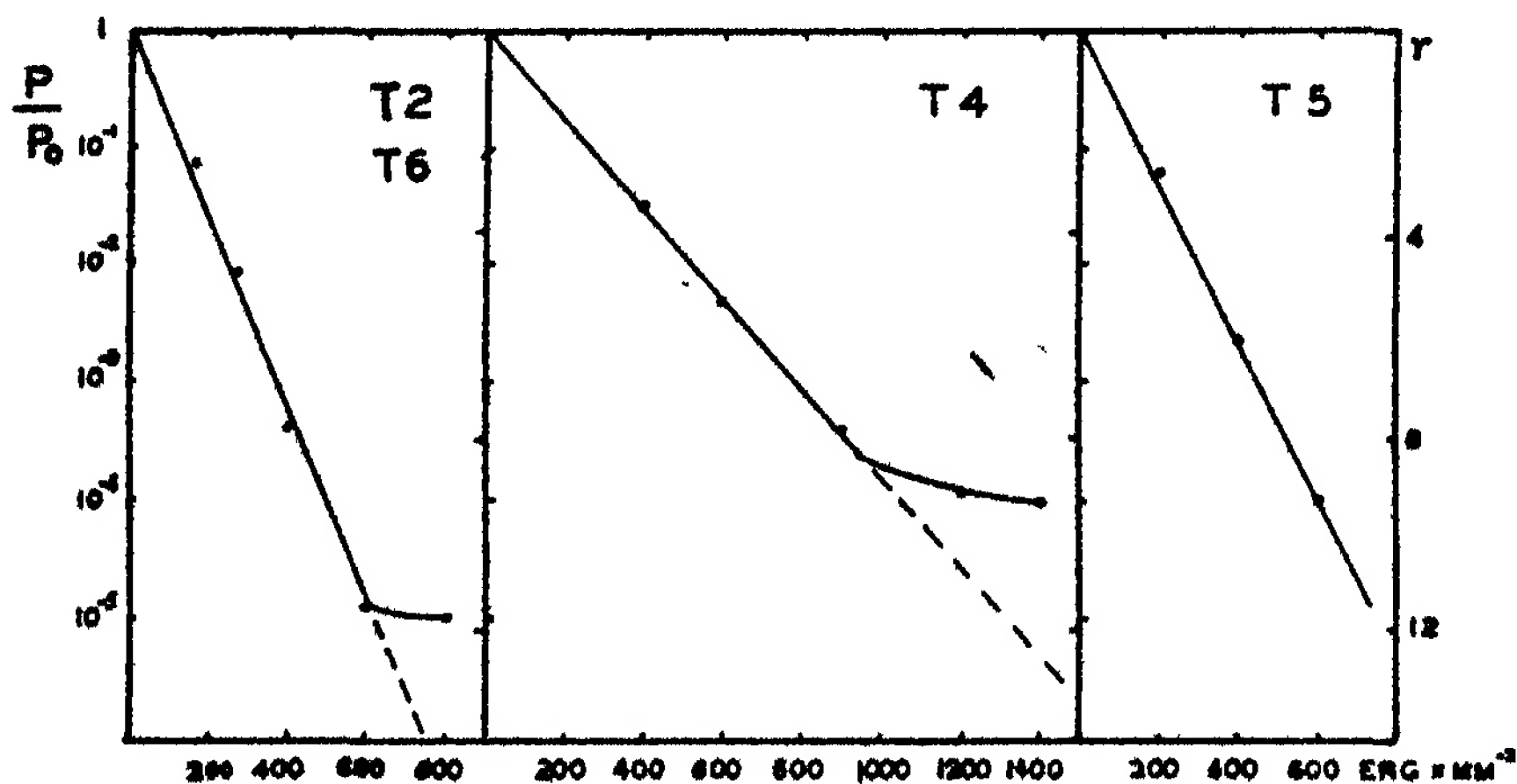


FIGURE 1

Survival curves for phages T2, T6, T4, T5 in synthetic medium.  $P/P_0$  = proportion of active phage particles after irradiation.  $r = \ln P_0/P$  = average number of lethal hits per particle. The deviations for high doses are due to the reactivation (described in this paper) taking place on the assay plates.

each bacterial cell that adsorbs one phage particle fails to divide and to produce a colony after plating. Incidentally, this fact makes it possible to calculate the number and rate of adsorption of ultraviolet inactivated phage particles. The unknown reactivating "factor" must be something that, when acting on a bacterial cell that has adsorbed an inactive phage particle, causes the production of active phage.

We first established that reactivation only occurs for the large-particle phages T2, T4, T6, T5, and their mutants, not the small phages T1 and T7. It was found next that the "factor" is partially phage specific, in the sense that even a concentrated suspension of one phage can generally not reactivate the particles of another irradiated phage. This can be tested by preparing mixtures of host cells with two phages in various proportions, the irradiated phage whose reactivation is to be tested being so diluted that

little or no reactivation would take place in the absence of the other phage suspension. After a few minutes of contact, the mixtures are diluted and plated with bacteria sensitive only to the phage whose reactivation is being tested. Since all phage suspensions are lysates of the same host cells, the specificity of reactivation suggests that the "factor" is not of bacterial origin.

An important exception to specificity is that cross-reactivation occurs between suspensions of phages of the T-even group (T2, T4, T6). These phages, although representing different wild types and distinguishable by a number of characteristics, are known to be serologically related and morphologically similar. Moreover, particles of these phages are known to be capable of mutual transfer of hereditary characteristics when adsorbed by the same host cell.<sup>6</sup> Reactivation of one of these T-even phages can be induced both by irradiated and non-irradiated suspensions of any of the other T-even phages.

These results suggested that the reactivating "factor" might simply be phage itself, in the sense that an inactive phage particle, if adsorbed on the same cell with another particle of the same or of a related strain, could be reactivated by transfer from the latter of the genetic locus or loci at which lethal mutations had occurred.

This hypothesis was qualitatively supported by the finding that cross-reactivation between two related phages can only occur in the presence of bacteria capable of adsorbing both phages, not in the presence of bacteria sensitive only to one of them. For example, T6 can reactivate T2 in presence of strain B, sensitive to both, but not in presence of bacteria B/6, sensitive to T2 and resistant to T6.

That reactivation is not caused by some other "factor" in the lysates was also supported by the finding that reactivation occurred with phage that had been purified from extraneous material by differential centrifugation (Strain T4r, kindly supplied by Dr. T. F. Anderson).

For quantitative testing, our hypothesis can be formulated more exactly by stating that reactivation should only occur inside bacterial cells that adsorb two or more bacteriophage particles (multiple-infected bacteria). This expectation can be tested for each phage by using several mixtures of bacteria and irradiated phage in different proportions, and calculating for each mixture the number of bacteria that adsorb more than one phage particle. If the average number of phage particles adsorbed per cell is  $x$ , the proportion of cells with more than one phage particle is given by the expression:  $1 - (x + 1)e^{-x}$ . For  $x$  small, this expression corresponds very closely to the proportion of cells with two phage particles. The values for multiple-infected bacteria thus obtained can then be compared with the actual numbers of bacteria that liberate phage in each mixture. These numbers are measured by plating a sample for phage plaque count before lysis

TABLE I

RELATION OF THE NUMBER OF BACTERIA YIELDING PHAGE TO THE NUMBER OF MULTIPLE-INFECTED BACTERIA

Series of mixtures containing the same number of bacteria and different numbers of phage particles were kept 10 minutes at 37°C., then plated for plaque count. The number of multiple-infected bacteria in each mixture was calculated from the formula

$$[B > 1P] = [1 - (x + 1)e^{-x}][B],$$

where  $[B]$  = total number of bacteria per ml.;  $[B > 1P]$  = multiple-infected bacteria per ml.;  $x$  = ratio "adsorbed phage/bacteria" in each mixture.

Expt. No. .... 41										43				38			
Flage..... T4										T4				T6			
Dose, $\text{cm}^3 \times \text{mm.}^{-1}$ ..... 800										900				500			
$x$	$[B > 1P]$ (Calc.)	Count (Exper.)	Ratio $1/y$ (Calc.) (Exper.)		$x$	$[B > 1P]$ (Calc.)	Count (Exper.)	Ratio $1/y$ (Calc.) (Exper.)		$x$	$[B > 1P]$ (Calc.)	Count (Exper.)	Ratio $1/y$ (Calc.) (Exper.)				
0.67	$1.7 \times 10^8$	$9 \times 10^7$	1.9		0.95	$1.9 \times 10^8$	$1.7 \times 10^7$	11		0.62	$1.6 \times 10^8$	$3.9 \times 10^7$	4.1				
0.33	$5.0 \times 10^7$	$3 \times 10^7$	1.8		0.48	$6.5 \times 10^7$	$5.4 \times 10^6$	12		0.31	$3.6 \times 10^7$	$1.2 \times 10^7$	3.0				
0.135	$1.1 \times 10^7$	$6 \times 10^6$	1.9		0.24	$1.9 \times 10^7$	$1.5 \times 10^6$	13		0.155	$1.3 \times 10^7$	$3.2 \times 10^6$	4.1				
0.067	$2.6 \times 10^6$	$1.3 \times 10^6$	2.0		0.09	$3.2 \times 10^6$	$2.2 \times 10^5$	14		0.062	$2.4 \times 10^6$	$6.7 \times 10^5$	3.6				
0.033	$6.8 \times 10^5$	$3 \times 10^5$	2.1		0.05	$9.0 \times 10^5$	$8.0 \times 10^4$	11		0.031	$5.4 \times 10^5$	$1.3 \times 10^5$	4.2				
0.013	$1.7 \times 10^5$	$1 \times 10^5$	1.7					/		0.015	$1.7 \times 10^5$	$4.1 \times 10^4$	4.1				
		Av.	1.92				Av.	12.2				Av.	3.85				
		$\sigma$	0.16				$\sigma$	1.2				$\sigma$	0.46				

occurs; every bacterium that liberates active phage will produce one plaque.

If the hypothesis is incorrect, the plaque counts should be directly proportional to the numbers of multiple-infected bacteria, the factor of proportionality depending on the dose of irradiation in a way that will be discussed later. A large number of experiments of this type gave results agreeing remarkably well with the expectation. Representative results are given in table 1. It was also found in these experiments that bacteria infected with more than one inactive phage particle liberate a roughly normal yield of fully active particles.

Our hypothesis can now be elaborated to give expectations for the actual values of the factor of proportionality between the numbers

of multiple-infected bacteria and of bacteria that yield active phage. We shall assume that a phage particle contains a certain number of different self-reproducing "units" (loci), each capable of undergoing a lethal mutation under the action of radiation. Reactivation depends on transfer of these units, the requirement for reactivation being that a given locus does *not* carry a lethal mutation in *all* of the particles that infect the same cell. The probability that this requirement is satisfied will depend on the dose of radiation, since the probability that a lethal hit takes place at the same locus in all infecting particles will increase with increasing doses of radiation. This hypothesis is graphically described in figure 2. It can be formulated mathematically by assuming that there are in each particle of a given phage  $n$  units each capable of giving a lethal mutation. We further assume, in first approximation, that the sensitivity of all units to radiation is the same.

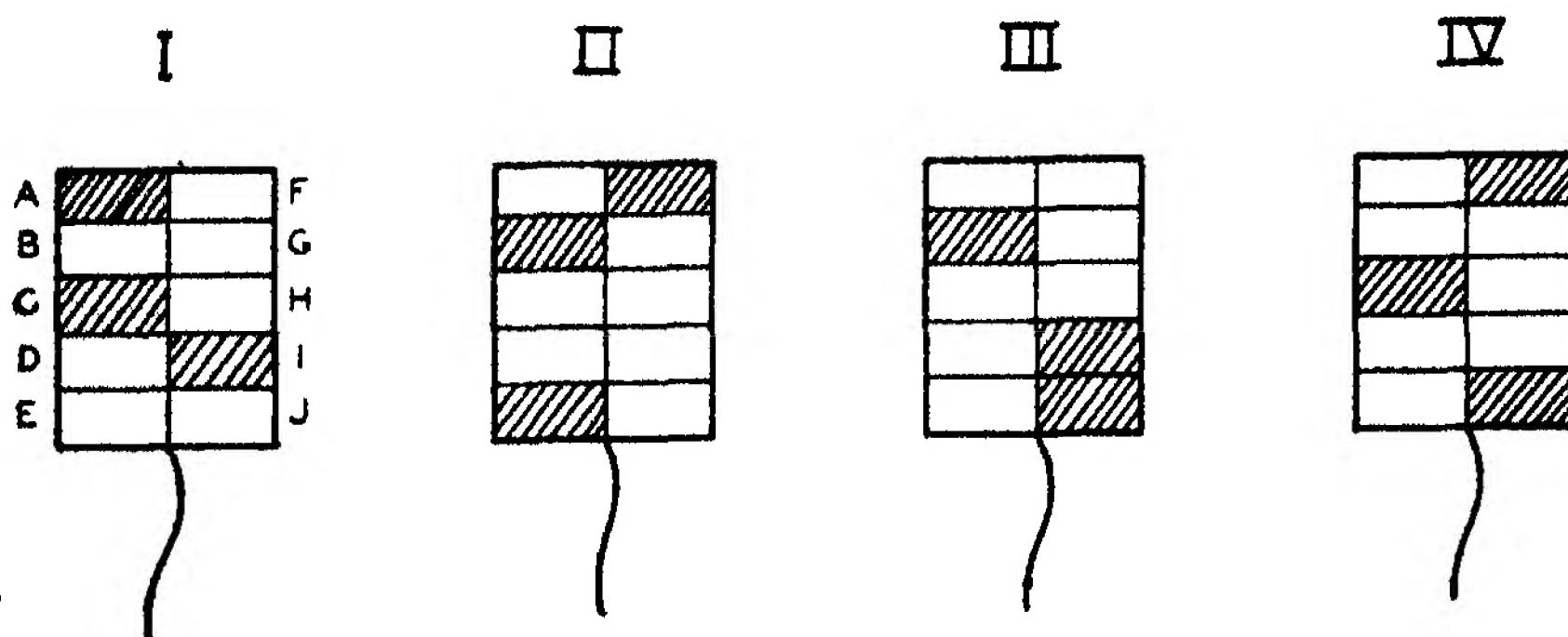


FIGURE 2

Schematic representation of phage particles, each carrying three lethal mutations distributed at random among ten loci.

A bacterium infected with particles (I + II) will yield active phage.

A bacterium infected with particles (I + III) will not yield active phage.

A bacterium infected with particles (I + IV) will not yield active phage.

A bacterium infected with particles (III + IV) will not yield active phage.

A bacterium infected with particles (I + III + IV) will yield active phage.

The probability  $y_k$  that none of the  $n$  units carries a lethal mutation in all of  $k$  particles adsorbed by the same cell is then found<sup>7</sup> to be

$$y_k = [1 - (1 - e^{-r/n})^k]^n \quad (1)$$

where  $r$  is the average number of lethal hits or mutations per particle. The value of  $r$  can be read directly from the regular survival curve, as given in figure 1,  $r$  being the natural logarithm of the ratio between the initial titre and the survival:  $r = \ln P_0/P$ .

For  $r/n \ll 1$ —that is, for low doses of radiation—the formula (1) can be simplified with good approximation to



$$y_k \approx e^{-r^k/n^{k-1}} \quad (1')$$

In particular, for bacteria that adsorb only two phage particles ( $k = 2$ ), the probability becomes

$$y_2 = [1 - (1 - e^{-r/n})^2]^n \quad (2)$$

$$y_2 \approx e^{-r^2/n} \quad (2')$$

It must be noticed that for low doses, when the probability that a lethal mutation at any given locus occurs in all particles is small, the values of  $y$  should tend to one if the efficiency of the transfer mechanism is complete, that is, if there is full recombination of active units to form active phage particles.

The formulas (1, 1') and (2, 2') can be tested experimentally by studying the dependence of the probability of reactivation (see table 1, columns 1/ $y$ ) on the dose of radiation. In particular,  $y_2$  can be obtained from experiments with bacteria in such excess that the probability of infection higher than double can be neglected. From the values of  $r$  and  $y$ ,  $n$  can be determined. If our hypothesis is correct,  $y$  should tend to one for low doses, and the values of  $n$  obtained from all experiments should be constant for each phage. A large number of experiments gave results agreeing with these

TABLE 2

CALCULATION OF THE NUMBER OF "UNITS" PER PHAGE PARTICLE (EXPERIMENTS WITH LOW DOSES)

$r$  = lethal hits per particle;  $y$  = proportion of multiple-infected bacteria that yield phage;  $n$  = number of units per particle calculated from the formula

$$y = [1 - (1 - e^{-r/n})^2]^n \approx e^{-r^2/n} \text{ (for } r/n \ll 1). \quad n = r^2/\ln(1/y).$$

PHAGE	$r$	1/ $y$	$n$	PHAGE	$r$	1/ $y$	$n$
T2-T2r	2.6	1	..	T6	3.6	1	..
	4.8	1.6	49		7.9	3.4	51
	7.7	3.5	47		9.4	5.7	50
	11.0	19	41		11.7	17	49
	14.3	93	45		12.6	40	48
		Av.	45.5		15.5	70	57
						Av.	50
T4-T4r	2.8	1	..	T5	2.8	1.9	12
	5.1	2.8	26		6.1	16	13
	7.8	5.7	36		9.2	67	19
	10.0	21	33			Av.	15*
		Av.	32				

\* Values uncertain, due to very low adsorption rate of this phage.

expectations. Some experiments are shown in table 2, where 1/ $y$  is given instead of  $y$  for convenience. We may then conclude that active phage is

formed from inactive particles by a highly efficient mechanism of transfer of any one of a relatively large number of independently transferable units: 45–50 for phages T2 and T6, 30–35 for phage T4, possibly around 15 for phage T5.

Formula (1) requires that the probability of reactivation increases with increasing values of  $k$ , that is, of the number of phage particles adsorbed per bacterium. This was verified by experiments in which bacteria were mixed with larger amounts of irradiated phage ( $x$  higher),<sup>8</sup> so that the probability of three or more phage particles being adsorbed by the same cell became appreciable. Representative data are given in table 3, and are seen to agree with the expectation that the values of  $y$  increase with increasing values of  $x$  (and of  $k$ ).

TABLE 3  
DEPENDENCE OF THE PROBABILITY OF REACTIVATION ON THE MULTIPLICITY OF INFECTION

DOSE, ERG $\times$ MM. $^{-1}$ .....		400	600	800	1000
EXPT. NO. $\nearrow$	PHAGE	$x$	$1/y$	$1/y$	$1/y$
71	T6	0.11	..	8.1	41
		0.55	..	7.8	25
		1.1	..	4.1	18
		2.7	..	5.1	15
77	T2	0.39	2.6	10.6	60
		0.78	2.4	9.3	35
		3.9	2.0	3.4	10
		7.8	1.65	2.15	5.3

These data permitted us to obtain a rough independent estimate of  $n$  from the probability of reactivation in bacteria with more than two phages. The values thus obtained for  $n$  are in sufficient agreement with those given in table 2, although generally a little lower. This systematic deviation seems to indicate that the efficiency of transfer in the case of infection with more than two particles is lower than 100%.

Our next question is the following: Does all inactivation of phage particles by ultraviolet light occur through production of lethal mutations in transferable units, or will some other mechanism of inactivation appear for very high doses? This point was investigated by using heavy doses (up to 40–50 lethal hits per particle) and comparing the results with the expectations from formula (2) for double infection. Any additional mechanism of inactivation appearing at high doses should result in a systematic deviation in the sense of less reactivation than predicted by the formula. No such deviation was found; this indicated that, even for large doses of radiation, all effect can be accounted for by lethal mutations in the transferable units.

This conclusion also has another interesting feature: it indicates that there is no appreciable amount of "linkage" in the transfer process. Any tendency of units to be transferred in groups rather than individually should give a deviation in the direction of too low reactivation at high doses, when the probability of producing two or more lethals within a linkage group becomes appreciable.

The next step was an attempt to elucidate the mechanism of transfer. The obvious analogy with crossing-over might suggest that the two entering particles exchange units directly, before any multiplication takes place, and that what then multiplies is a fully active particle resulting by exchange. This possibility is difficult to reconcile with the very high efficiency of reactivation: in case of crossing-over we would hardly expect that all active units be collected into one active particle. Another test can be made by the cross described in figure 3. Remembering that an active particle of one phage may reactivate an inactive particle of another phage, we may expect that transfer will also occur between active and inactive particles of the same phage. If there is direct crossing-over, an active particle, if adsorbed with inactive ones onto the same cell, should often be inactivated by transfer of some "lethal" unit.

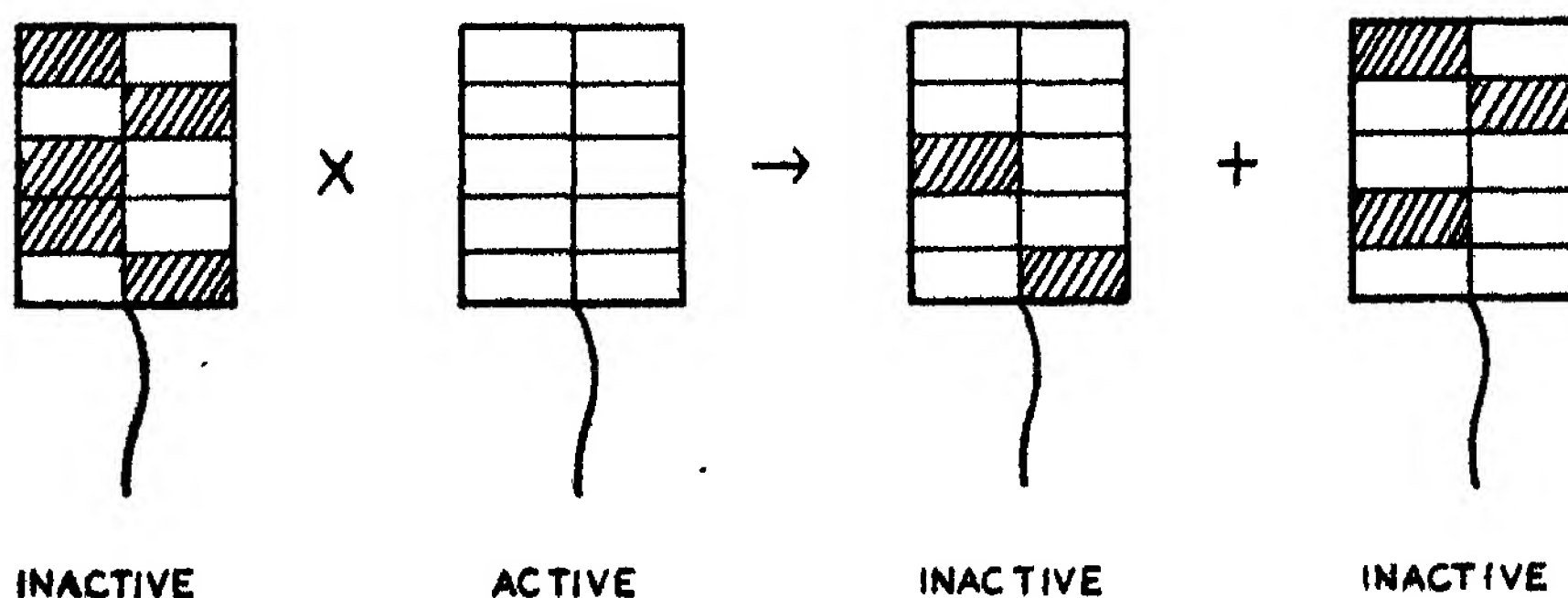


FIGURE 3.

Schematic representation of the expectation in case of "cross" between a heavily irradiated particle and an active particle, assuming transfer of units by direct crossing-over between the two infecting particles.

In the actual experiments, phage suspensions were given large doses of radiation (20–50 hits per particle). Mixtures were set up containing bacteria, inactive phage, and active phage in proportion such that a large number of cells received one active and one or more inactive particles. If there were direct exchanges before reproduction, the result should be the one described in figure 3. A high proportion of the infected cells should end up with two inactive particles and, therefore, should yield no active

phage. This was not found to occur in any case: an active phage particle was never inactivated by "crossing" with an inactive one."

A possible alternative interpretation of the transfer is that each of the active units can reproduce copies of itself in excess of the number needed for multiplication of the phage particle as a whole. The copies of each unit might then either become incorporated into other phage particles that missed them, or come together to reconstitute active phage particles, independently of the origin of the individual units from one or another of the infecting particles. We have no evidence as yet available whether the "lethal" units do not multiply, or multiply in a way that makes them unfit for their niche in the phage particle. In view of the high efficiency of the reactivation process and of the approximately normal yield of phage particles, it seems certain that, if the inactive units reproduce at all, they do not have any appreciable chance of becoming incorporated into the newly formed particles.

We also have no evidence as yet as to whether the active units in an inactive particle will proceed to multiply in single-infected cells. The same may be said for the question whether the active units remain in spatial relation to each other, as it were, in a "frame phage particle," while reproducing excess copies that are incorporated into the new particles that are formed. Work to attack some of these problems is now in progress.

A point of considerable interest is that in numerous experiments in which phage particles were inactivated by x-rays instead of ultraviolet light, no reactivation occurred, although the x-ray inactivated particles were still adsorbed by the bacterial cells.

*Discussion:* These experiments have suggested a possible mechanism of reproduction of phage particles inside the host cell, according to which reproduction would take place, as it were, in an "atomistic" way, by independent reproduction of a number of units and incorporation of these into the final phage particles. A number of problems may be raised, as to the nature of the self-reproducing units, the structure of the phage particle, and the relation of its mechanism of reproduction to that of other self-reproducing entities (genes, plasmagenes).

In the first place, each unit seems to react as a distinct photochemical entity in respect to ultraviolet quanta. The assumption of equal sensitivity is very likely unjustified; a variation in sensitivity from locus to locus would tend to make the calculated number of loci per particle too small, since the most sensitive units have a greater chance of being lethally hit earlier in all infecting particles. This would result in less reactivation and apparently lower number of loci. Our estimates of the number of loci are therefore minima. It is interesting to notice that phage T4, which is about twice as resistant to ultraviolet than the related phages T2 and T6, also appears to have fewer loci. Its higher resistance seems, therefore, to be due

to actual absence of a number of loci rather than to absence of some particularly sensitive locus.

Our results with the small phages T1 and T7, where no reactivation was detected, do not prove that these phages do not possess a number of independently transferable loci. They only indicate that, if such loci occur, they are too few to be detected in our experiments, that is, fewer than 8 or 10. These phages are actually about 2.5 times more resistant to ultraviolet than phage T4.

As for the failure of particles inactivated by ionizing radiation (x-rays) to undergo reactivation, this may be interpreted as indicating, either spread of the lethal effect of each ionization (or group of ionizations) to a large number of loci, or, less likely, an ability of the x-rays to cause other changes than those produced by ultraviolet. A projected study with various monochromatic radiations may throw some light on this question. It is interesting to notice that Lea and Salaman,<sup>10</sup> extending earlier work on the rate of inactivation of phage by ionizing radiation,<sup>11</sup> and analyzing their results in terms of the density of ionization, recently concluded that the "sensitive zone" of a large phage particle may be resolved into about fourteen units (genes?). Because of the assumptions involved in their calculations, their results by themselves can hardly be considered as more than qualitative indication of a geometrically complicated organization of the radiation sensitive material of the phage particle. In the light of our experiments, however, the interpretation offered by Lea and Salaman appears quite plausible, at least in assuming a multiplicity of sensitive units.

The incorporation of discrete units into organized phage particles is not easy to visualize, in view of the complex structure and morphology of the latter.<sup>12</sup> We can imagine that each active unit impresses its specificity on a number of elements produced in excess inside the host cell, elements which represent the raw material then utilized to build more phage particles. The units carrying lethal mutations may be unable to mold the substrate in their own image and likeness.

It is plausible, although not yet experimentally demonstrated, that some of the transferrable units that can undergo lethal mutations are the same gene-like entities responsible for determination of the transferable characters studied by Delbrück and Bailey<sup>9</sup> and by Hershey<sup>13</sup>.

The mechanism of transfer suggested for bacteriophages appears to differ from that of crossing-over by being a transfer of units by "infection" rather than an "exchange" of portions of gene strings. One might, however, conceive that excess gene copies similar to the copies of our units are also produced in the cell nucleus, but have no opportunity of being incorporated by "infection" into the chromosomal continuum because of a more rigid integration of the genes in the chromosome (needed to meet the requirements of the sexual process) than of the units in the virus particle. Excess pro-



duction of full or partial gene copies has been suggested both on the basis of cytological studies<sup>13</sup> and in connection with theories of gene action.<sup>14</sup>

The transfer mechanism appears to represent a novel, and, in its way, rather efficient means of effecting genetic recombination between virus particles.

Our results thus offer direct evidence for interpreting inactivation of viruses by radiation as due to lethal mutations, and strengthen the concept of a fundamental similarity between viruses and the genetic material of other organisms. They indicate that a virus particle may rather be comparable to a gene complex than to an individual gene. A virus particle can undergo a number of independent mutations,<sup>2, 15</sup> a property that may be shared by certain "gene complexes," defined as cross-over units resolvable into two independently mutable units.<sup>18</sup> The independent mutability of these subgenic units is, however, somewhat doubtful.<sup>16</sup> The virus particle might better be compared to a chromosome, although it behaves more as a unit toward x-rays than the latter. The self-reproducing components seem to possess in the virus particle a degree of solidarity intermediate between that of the components in the supposed gene complex and that of the genes in the chromosome.

There are obvious similarities between the transfer phenomenon here described and other phenomena in which a determinant of heredity is transferred into a new genetic complex, as in the case of type transformation in pneumococci.<sup>17</sup> Phage reproduction may be a particularly favorable material on which to analyze the mechanisms involved.

One interesting application is the possibility of analyzing differences between related but independent wild-type phage strains which can be "crossed" (like the T-even group) in terms of differences in the number of units that can be exchanged (shared loci). Preliminary work in this direction appears promising.

*Summary.*—Inactivation of large bacteriophages by ultraviolet light is due to lethal mutations at a number of different loci. Each of these loci appears to be independently transferable from one phage particle to others inside the same bacterium. The transfer of loci between irradiated inactive particles results in formation of active phage if the infecting particles, taken as a group, possess at least one copy of each locus in active form. The number of loci can be calculated for each phage, and is at least 30 to 50 for some of them. Phage growth appears to take place by independent reproduction of each of these "unit loci" inside the host cell.

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- <sup>6</sup> Delbrück, M., and Bailey, W. T., Jr., *Cold Spring Harbor Symp.*, 11, 33 (1946).
- <sup>7</sup> Formula (1) was derived as follows:  
 Probability that a given locus receives no lethal mutation =  $e^{-r/n}$ .  
 Probability that a given locus receives a lethal mutation =  $1 - e^{-r/n}$ .  
 Probability that a given locus receives a lethal mutation in all of  $k$  particles =  $(1 - e^{-r/n})^k$ .  
 Probability that a given locus does not receive a lethal mutation in all of  $k$  particles =  $1 - (1 - e^{-r/n})^k$ .  
 Probability that none on  $n$  loci receives a lethal mutation in all of  $k$  particles =  $y_k = [1 - (1 - e^{-r/n})^k]^n$ .
- <sup>8</sup> It should be noticed that  $x$  (average number of phage particles adsorbed per bacterium) is different from  $k$  (actual number of phage particles adsorbed by a given bacterium). The proportion of cells with  $k$  particles is  $(x^k/k!) e^{-x}$ .
- <sup>9</sup> These results differ from earlier observations<sup>8</sup> of interference between inactive and active particles of the same phage. The older experiments, however, were done under conditions not comparable with those discussed here.
- <sup>10</sup> Lea, D. E., and Salaman, M. H., *Proc. Royal Soc., B*, 133, 434 (1946).
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- <sup>12</sup> Luria, S. E., and Anderson, T. F., *Ibid.*, 28, 127 (1942).
- <sup>13</sup> See Schultz, J., *Colloid Chemistry*, 5, 819 (1944).
- <sup>14</sup> See Emerson, S., *Ann. Missouri Bot. Garden*, 32, 243 (1945).
- <sup>15</sup> Luria, S. E., *Genetics*, 30, 84 (1945).
- <sup>16</sup> Stadler, L. J., *Ibid.*, 31, 377 (1946).
- <sup>17</sup> See McCarty, M., *Bact. Rev.*, 10, 63 (1946).
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## NUTRITIONAL LIFE HISTORY AS INFLUENCED BY DIETARY ENRICHMENTS. I.\*

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Starting with a basal diet of natural foods which is adequate in the sense and degree that rat families are thriving upon it in the 63rd generation, the effects of enriching the diet (*a*) with protein in the form of poultry meat, and (*b*) with added calcium also, are being studied.

These enrichments in protein increase the rate of growth and result in earlier puberty. With females, however the records from puberty to middle age are variable when the dietary is enriched in protein food only. They raise the question whether the more rapid growth and earlier sexual

maturity are advantageous to all individuals, when induced by dietary enrichment essentially in protein alone. That the average differences thus induced are significant is shown by the data of tables 1 and 2.

TABLE 1  
AVERAGE DIFFERENCES IN BODY WEIGHTS OF RATS ON BASAL DIET ALONE AND WITH ADDED PROTEIN (10 RATS IN EACH CASE)

AGE, DAYS	ON BASAL DIET (DIET 16) ONLY, GRAMS	ON BASAL + PROTEIN (DIET 16P5) GRAMS	DIFFERENCE ± ITS P. E.* GRAMS	CRITICAL RATIO
MALES				
28	49.9 ± 1.5*	49.1 ± 1.4	0.8 ± 2.1	0.4
35	58.1 ± 2.1	67.1 ± 2.5	9.0 ± 3.3	2.7
56	98.4 ± 3.8	130.6 ± 2.9	32.2 ± 4.8	6.7
84	153.9 ± 4.9	202.2 ± 3.2	48.3 ± 5.9	8.2
FEMALES				
28	46.4 ± 1.5	46.8 ± 1.4	0.4 ± 2.1	0.2
35	54.0 ± 1.9	61.2 ± 2.1	7.2 ± 2.8	2.6
56	86.1 ± 2.4	108.2 ± 1.9	22.1 ± 3.1	7.1
84	119.5 ± 2.4	158.5 ± 2.9	39.0 ± 3.8	10.3

\* This ± precision measure is the classical Probable Error.

With both sexes it is clear that the data of table 1 show equivalence at the starting point of 28 days of age (conventional "end of infancy" in the rat) and increasing divergence, due to the greater growth on the diet of higher protein content, up to the age of 84 days. Beyond that age the difference diminishes as the animals on both diets are approaching the normal average adult size.

Table 2 shows the mean differences in several aspects of the early breeding records of females on the two diets. At every point the extra protein has here accelerated the result.

TABLE 2  
AVERAGE RECORDS OF 10 FEMALES EACH ON BASAL DIET ALONE AND WITH ADDED PROTEIN. ALL THESE RECORDS ARE TO THE AGE OF 210 DAYS ONLY.

	ON DIET 16 (BASAL ONLY)	ON DIET 16P5 (ADDED PROTEIN)
Age at birth of first young, days	132	98
Total number of litters borne	19	28
Total number of young borne	123	174
Young reared to the age of 28 days	70	146
Total weight of young at 28 days of age, grams	2481	5786
Average weight of young at 28 days of age, grams	35.4	39.6

The foregoing are representative of the majority of our experiments, with matched groups of litter-mate controlled animals, showing accentuation of growth and of early reproduction as results of the enrichment of the basal ration with protein in the form of poultry meat.

In another comparison of the same kind, however, three out of thirteen females have broken down in or after early reproduction and with the development of a nervous condition suggestive of relative calcium deficiency. Autopsies of two of them showed no major infections while chemical analysis of the third showed only 0.68 per cent of body calcium upon death at 169 days as compared with averages of 0.95 to 1.15 per cent found in animals of the same age which had subsisted on the basal diet without supplement. The occurrence of such a low calcium condition is confirmed in other analyses of experimental animals from comparable diets.

In a further series of experiments, the same protein supplement is being fed with basal diets of different calcium contents. The rat families of more liberal calcium intake have been (and are) entirely free from any sign of a period of imbalance, and show higher vitality than their litter-mates and cousins on lower-calcium dietaries. This may or may not appear in the numerical data of growth and development; but is visible in their alertness and general appearance, and palpable in their superior muscle tone and firmness of subcutaneous tissue.

Thus a protein enrichment which, when added alone to a rather low calcium diet, may merely stimulate growth and early puberty at some cost of good balance and stability of the nervous system, appears to be freed from this hazard of imbalance when the basal dietary is of higher calcium content.

( ) Experiments with diets of both the above-described types are being continued, and we hope to report later upon their relations to nutritional well-being in the later segments of the life cycle.

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## *FURTHER STUDIES OF THE INFLUENCE OF NUTRITION UPON THE CHEMICAL COMPOSITION OF THE BODY\**

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Previous papers from this laboratory have described experiments with rats fed a basal diet consisting of natural foods (Diet A: Laboratory No. 16) which diet has shown itself adequate to the support of normal health, growth, and reproduction generation after generation, yet can be improved either by shifting the proportions of foods it contains or by enrichment with certain specific nutrients.<sup>1-4</sup> Independently of other change in this basal diet, addition of calcium<sup>2-4</sup> or of vitamin A increases the body's con-

tent of the respective nutrient and also improves the nutritional life history.

Corresponding enrichments of the same basal diet with riboflavin and protein might, however, be expected to act interdependently (rather than independently), consistently with the view that the body holds riboflavin in combination with protein, and with the general mass-action or concentration principle. This has been found to be the case.

In one series of five comparisons, rats of the same genetic and nutritional background were fed diets alike in other respects and containing the same liberal amount of riboflavin (7 mcg. per gram of air-dry food mixture) at two levels of protein intake. The average results were: (a) with 12 per cent of protein in the food, 5.70 mcg. of riboflavin per gram, and 16.95 per cent of protein, in the body; (b) with 32 per cent of protein in the food, 6.63 mcg. of riboflavin per gram, and 18.42 per cent of protein, in the body. Thus when the protein content of the food was varied as widely as from 12 to 32 per cent, the increased protein intake raised the body's concentration levels of both protein and riboflavin by about one-tenth. These findings thus confirm and extend those of other investigators<sup>6, 7</sup> whose experimental work, largely simultaneous, entered the field at different angles of approach from ours.

In other experiments to be reported in full elsewhere, we have compared the effects of diets containing, respectively, 1, 3, and 9 mcg. of riboflavin per gram. When the protein content of the diet was 12 per cent, the percentages of body protein found at these three riboflavin intake levels were 16.41, 16.90, and 17.22, and the amounts of riboflavin per gram of body were 6.11, 6.95, and 8.14 mcg., respectively. When the diet contained 20 per cent of protein the effect of the same variation of riboflavin intake upon body composition was less. The respective percentages of body protein were 18.00, 18.20, and 18.60, indicating that at an intake level of 20 per cent of food-protein, the body-protein content was practically upon its plateau level. At this same protein intake level, the corresponding amounts of riboflavin in the body were 6.04, 6.67, and 7.17 mcg. per gram, indicating that the plateau level of the body's riboflavin content was reached with intake levels of about 3 to 7 mcg. per gram of food according to the experimental conditions.

In contrast to the mutually favorable effects of protein and riboflavin, the acceleration of growth by relatively high protein food may result in retardation of the body's normal developmental gain in calcium content. This was noticeable in our experimental enrichment of a basal diet of about minimal adequate calcium content by the addition of meat, and has also been found in parallel enrichments with pure casein so that it appears to be essentially a protein effect. Or it may be regarded as an effect of enhanced growth, which in these cases was induced by the feeding of extra protein.

Further experiments upon the relation of protein-augmented growth to the calcium content of the body at different ages are in progress and we plan to publish additional findings later. We have, however, no desire to "reserve" this field research. Rather, we are publishing this brief account now in the hope that other investigators of high protein feeding may study further, and from various angles, the indication that the body's ability to make good use of high protein diets depends largely upon such diets possessing also a liberal calcium content.

\* Aided by grants from the Carnegie Institution of Washington and from the John and Mary R. Markle Foundation.

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## CYTOLOGICAL PHENOMENA AND SEX IN *HYPOMYCES SOLANI* F. *CUCURBITAE*

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*Hypomyces solani* f. *cucurbitae* S. & H. is a hermaphroditic, self-incompatible fungus (Hansen and Snyder, 1943). However, male and female strains of the same organism have also been found. In a recent paper (1946) Hansen and Snyder describe the interesting sex-behavior of this fungus.

Perithecia will be produced only when conidia from either a male or a hermaphroditic strain are placed on perithecial primordia of either a female or a hermaphroditic culture of the opposite compatibility group. The male strain differs from both the female and hermaphrodite by the absence of perithecial primordia and sporodochia, while the female, although resembling the hermaphrodite in cultural characters, cannot act as the fertilizing agent.

When the different crosses are analyzed by means of single ascospore cultures it is found that the progeny of the combination, hermaphrodite  $\times$  male, consists of 50% males and 50% hermaphrodites. The same 1:1



relationship holds for the ascospore progeny of the cross, female  $\times$  hermaphrodite, while the combination, hermaphrodite  $\times$  hermaphrodite, gives hermaphrodites only. However, when a female is crossed with a male, the progeny will show, apart from the parental types, a certain number of hermaphrodites and a certain number of neuters. The latter are strains which are completely unable to react sexually either as female or male. These unexpected types, hermaphrodites and neuters, appear in about the following ratio: 3 males: 3 females: 1 hermaphrodite: 1 neuter. This phenomenon was explained (Hansen and Snyder, 1946) by the assumption that the genes for male and female sex are not alleles but occupy different loci in homologous chromosomes, and that crossing over takes place between them.

In the hope of obtaining a cytological explanation for these phenomena, the nuclear condition obtained in the various crosses was studied. Perithecia which had not yet begun to exude ascospores were fixed in a modified Carnoy's solution (1 part glacial acetic acid, 2 parts absolute alcohol, 3 parts chloroform) for at least 48 hours, then smeared in a drop of acetocarmine containing a liberal amount of iron. A coverslip was then added, the slide heated and the preparation pressed flat. Strains of the fungus differed considerably in their ability to take up the stain.

Fusion of the parental nuclei takes place in the young ascus, and as soon as the latter begins to enlarge the fusion nucleus begins its meiotic division. The prophase is protracted and reaches its close only when the ascus has attained nearly full size. The prophase chromosomes are large compared with their small metaphase size, but since they are entangled and do not stain well it was only in a few cases that their number could be definitely determined at the prophase stage. In these cases, however, something of their morphology could also be made out. Beginning with the first prophase the chromosomes become progressively smaller; in the second metaphase they are already very minute, but since the chromosomes in these later stages stain better they could more readily be counted.

It was found that in the cross female  $\times$  male the diploid nucleus has three pairs of chromosomes which can easily be distinguished. The two members of the first pair are relatively large and slightly heterobrachial; those of the second are a little more than half as long and those of the third about one third the size of those of the second. The largest chromosome has a satellite region which in some prophase preparations appears to be attached to the nucleolus.

However, when the cross hermaphrodite  $\times$  hermaphrodite was investigated it was found that four pairs of chromosomes were present. One pair was relatively large, another very small, as in the above cross, but here there were two pairs of medium sized (chromosomes) which were essentially



alike, except that in some preparations the members of one pair appeared to be slightly curved while those of the other were straight.

The cross hermaphrodite  $\times$  male showed seven diploid chromosomes. In the first anaphase three of these migrated to one pole while the remaining four moved in the opposite direction. The same was seen in preparations of asci derived from fertilization of a female by a hermaphrodite.

Thus a hermaphrodite haploid would have four chromosomes (one large, two medium and one small), while a male or a female would have only three (one large, one medium and one small). But how do the hermaphrodite and the neuter types arise from the cross female  $\times$  male? In a few preparations it appeared that the six diploid chromosomes separated unequally, 4 going to one pole, only two to the other. This would mean that the two medium sized chromosomes, one of which contains the factor for male sex, the other for female, fail to separate in a certain number of cases (to judge from the genetical data) and both move to one pole. The spores formed from the nucleus containing only two chromosomes will be neuters.

The results obtained here provide an explanation for the derivation of male, female and even neuter clones from a hermaphroditic fungus. They also demonstrate that the haploid chromosome number in this fungus may be two, three or four.

These cytogenetic studies in *Hypomyces* are being continued by the writer.

\* The work reported here was done under the direction of Prof. H. N. Hansen and Prof. W. C. Snyder of the Division of Plant Pathology, University of California, Berkeley, California.

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## MULTIPLY VALUED HARMONIC FUNCTIONS. GREEN'S THEOREM

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1. *The Multiple-Leaved Domain.*—A multiple-leaved space  $\mathfrak{M}$  in three dimensions is the analog of a Riemann surface in the plane. Let  $T$  be a bounded domain on  $\mathfrak{M}$ , whose boundary consists of a finite number of closed branch curves  $s_{(1)}, \dots, s_{(r)}$  and a bounded exterior frontier  $T^*$ , in

such a way that the situation is equivalent topologically to an  $m$ -leaved sphere in which the  $s_{(i)}$  correspond to branch circles within the sphere, no two of which loop or have points in common. We assume that the  $s_{(i)}$  are of zero capacity, considered as closed point sets in space. For our purposes there is no essential loss of generality if we restrict ourselves to a single branch curve  $s$ , connecting cyclically all  $m$  leaves. We are specially interested in the rôle of the branch curve, and since the boundary  $T^*$ , as described by means of the topologically equivalent image space, is not involved by it but lies on  $m$  separate leaves of  $\mathfrak{M}$ , we may take the parts  $T_{(1)}^*, \dots, T_{(m)}^*$  of the boundary to be regular surfaces.

**THEOREM.** *Let  $u(M)$ ,  $v(M)$  be two functions, bounded and harmonic in  $T$  and on  $T^*$ . Then, with  $n$  as the exterior normal to  $T^*$  at  $P$ ,*

$$\int_{T^*} \left( u \frac{dv}{dn} - v \frac{du}{dn} \right) dP = 0, \quad (1)$$

$$\int_{T^*} u \frac{dv}{dn} dP = \int_T (\text{grad } u \cdot \text{grad } v) dM. \quad (2)$$

The essence of the theorem is that there is no contribution to the boundary integrals from the branch curves themselves. The proof of (1) is simpler than that of (2), although, of course, (1) is a consequence of (2).

In a paper which dealt primarily with multiple-valued Green's functions in the case where the branch curves were infinite straight lines, Sommerfeld<sup>1</sup> gave incidentally a proof of (2), assuming, however, that the branch curves were sufficiently smooth and that  $u(M)$  was continuous on the branch curves. But this limitation is awkward and disguises the essential nature of the theorem. Examples show that continuity on the branch curves cannot be specified in advance.<sup>2</sup> It is essential, however, that the function be bounded and that the branch curves be sets of zero capacity, because if either of these restrictions is eliminated the theorem is no longer valid. The behavior at the branch curves tends to be determined by the geometric character of the domain rather than by the individual function.

The multiple-leaved space  $\mathfrak{M}$  lies on a univalent base space, and the domain  $T$  lies on a univalent domain  $\bar{T}$  composed of all points whose coördinates  $(\bar{x}, \bar{y}, \bar{z})$  are the same as the coördinates of any point of  $T$ . We denote by barred symbols, in this way, the projection on the base space of a given configuration on  $\mathfrak{M}$ . A point  $Q$  of the branch curve  $s$  is defined to be a *limit point* of a set  $E$  of points on  $T + s$ , if  $\bar{Q}$  is a limit point of  $\bar{E}$ .<sup>3</sup> With this definition, it is seen that any infinite set on  $T + T^* + s$  has a limit point on  $T + T^* + s$ .

**2. Proof of (1).**—The development of (1) may be indicated briefly. Construct a sequence of approximating domains  $T_k$  to  $T$  with boundaries  $T^*$  (fixed) and  $t_k$  (variable), the latter being multivalent tori which isolate

the  $s$ . The  $t_k$  may be obtained as images of tori in the equivalent topological space, and then smoothed out into regular surfaces by a well-known process. We may now determine a corresponding sequence of functions  $u_k(M)$ ,  $v_k(M)$ , bounded and harmonic in  $T_k$ , and taking on the same values as  $u(M)$ ,  $v(M)$ , respectively, on  $T^*$  and zero values on  $t_k$ . These functions are constructed by means of a generalization of the Schwarz alternating process. Since their behavior at a point of  $t_k$  or  $T^*$  depends merely on these boundaries locally, they are entirely regular at such points, and Green's theorem may be applied to  $T_k$ . Hence

$$\int_{T^*} \left( u_k \frac{dv_k}{dn} - v_k \frac{du_k}{dn} \right) dP + \int_{t_k} \left( u_k \frac{dv_k}{dn} - v_k \frac{du_k}{dn} \right) dP = 0.$$

But the last integral vanishes, through the definitions of  $u_k$ ,  $v_k$ . Let then  $k$  become infinite. It is seen without difficulty that the  $u_k$ ,  $v_k$  converge to functions  $U(M)$ ,  $V(M)$  harmonic in  $T$  and on  $T^*$ , and bounded, the convergence being uniform in the neighborhood of  $T^*$ . Hence

$$\int_{T^*} \left( U \frac{dV}{dn} - V \frac{dU}{dn} \right) dP = 0.$$

But also it is seen that  $U$ ,  $V$  take on the same boundary values as  $u$ ,  $v$ , respectively, on  $T^*$ . We shall see that they are identical with  $u$ ,  $v$  in  $T$ , and in this way we shall obtain the identity (1).

In fact the function  $U-u$  vanishes on  $T^*$ , and is bounded and harmonic in  $T$ , and if it is not identically zero, either it or its negative is positive somewhere in  $T$ . Let  $w(M)$  denote this choice of  $U-u$  or  $u-U$ . Then  $w(M)$  has a positive upper bound  $B$  in  $T$ . We shall see that this is impossible on account of the hypothesis that  $s$  is of zero capacity.

3. *Kellogg's Theorem on the Upper Bound.*<sup>4</sup>—The theorem of O. D. Kellogg, on the capacity of sets on the boundary associated with the upper bound of a harmonic function, may be adapted to multiply valued functions, and, incidentally, applied to subharmonic functions.<sup>5</sup>

**KELLOGG'S THEOREM.** Let  $w(M)$  be subharmonic (in particular, harmonic) in the bounded domain  $T$  on  $\mathfrak{M}$  and possess the finite least upper bound  $B$  in  $T$ . With  $\epsilon > 0$ , let  $e$  be the set of boundary points  $Q$  (that is,  $Q$  on  $T^*$  or on a branch curve) where

$$\limsup_{M \rightarrow Q} w(M) \geq B - \epsilon, \quad M \text{ in } T.$$

Then the base set  $\bar{e}$  of  $e$  is closed and of positive capacity.

The proof follows closely the method of Kellogg. It will be noted that since the branch curves are of zero capacity the portion of  $e$  on the exterior boundary  $T_{(s)}^*$  for at least one of the leaves  $T_{(s)}$  must be of positive capacity. This fact insures the uniqueness of bounded harmonic functions

in general as determined by their boundary values on  $T^*$ , without regard to their values on the branch curves, and in particular completes the proof of (1).

4. *Proof of (2).*—We consider first the summability over  $T$  of  $(\text{grad } u)^2$  and the proof of the identity

$$\int_T (\text{grad } u)^2 dM = \int_{T^*} u \frac{du}{dn} dP. \quad (3)$$

The method used for (1) shows that

$$\begin{aligned} \int_{T^*} u \frac{du}{dn} dP &= \lim_{k \rightarrow \infty} \int_{T_k} (\text{grad } u_k)^2 dM \\ &= \int_{T_1} (\text{grad } u)^2 dM + \lim_{k \rightarrow \infty} \int_{T-T_1} (\text{grad } u_k)^2 dM \\ &= \lim_{j \rightarrow \infty} \int_{T_1} (\text{grad } u)^2 dM + \lim_{j \rightarrow \infty} \lim_{k \rightarrow \infty} \int_{T-T_1} (\text{grad } u_k)^2 dM, \end{aligned}$$

provided that we define  $u_k$ , for convenience, as zero in  $T-T_k$ . A similar identity holds for  $\int_{T^*} u \frac{dv}{dn} dP$ .

In particular the summability of  $(\text{grad } u)^2$  is established, and since  $2|\text{grad } u \cdot \text{grad } v| \leq (\text{grad } u)^2 + (\text{grad } v)^2$  the summability of  $\text{grad } u \cdot \text{grad } v$  is also verified. Moreover if (3) is proved it will follow that

$$\lim_{j \rightarrow \infty} \lim_{k \rightarrow \infty} \int_{T-T_1} (\text{grad } u_k)^2 dM = 0,$$

so that the corresponding limit involving  $\text{grad } u_k \cdot \text{grad } v_k$  will also vanish, and (2) will be proved.

Since we are dealing with a single branch curve  $s$  there is no loss of generality in assuming that all the branches  $l_{(i)k}$  of the tori  $l_k$  have the same base set  $l_k$ , and (by adjoining univalent domains) that all the branches  $T_{(i)}^*$  of  $T^*$  have the same base set  $T^*$ . The symmetric function,

$$V(\bar{M}) = \sum_{i=1}^m u_{(i)}^2(M),$$

will be bounded and univalent in  $T$ . Moreover, the Laplacian of  $V$  will be given by the formula

$$\nabla^2 V(\bar{M}) = 2 \sum_{i=1}^m (\text{grad } u_{(i)})^2,$$

$u_{(i)}(M)$  being harmonic, and  $\nabla^2 V(\bar{M})$  will be summable and  $V(\bar{M})$  subharmonic in  $T$ . Also  $dV/dn = 2 \sum u_{(i)} du_{(i)}/dn$ . The identity (3) therefore is equivalent to the identity

$$\int_{\bar{T}} \nabla^2 V d\bar{M} = \int_{\bar{T}^*} \frac{dV}{dn} dP. \quad (4)$$

It suffices to prove the latter.

We may now drop the bars in our notation, since henceforth we deal with univalent functions. The proof of (4) seems to involve considerable detail, but the main steps may be stated in terms of several lemmas. With  $\rho(P) = \nabla^2 V(P)/(4\pi)$  and  $T'$  a domain which includes  $T$  (the erstwhile  $\bar{T}$ ), but in which the properties of  $V(M)$  still hold, we define the function

$$V_1(M) = V(M) + \int_{T' \setminus MP} \frac{\rho(P)}{MP} dP$$

which is harmonic in  $T'$  and bounded below, since  $\rho(P) \geq 0$ .

LEMMA 1. *Let  $F$  be a closed set of positive capacity and exterior frontier  $t$ , surrounded by a domain  $T$  of exterior boundary  $S$ , the latter being a closed regular surface. There exist two mass distributions  $\mu(e)$  and  $\nu(e)$ , each of one sign, on  $t$  and  $S$ , respectively, whose combined potential takes on arbitrary constant values, namely,  $K$  at all points of  $S$ , and  $N$  at all points of  $t$  which are regular with respect to  $T$ . If  $N \neq K$  the distribution  $\mu(e)$  is not identically zero, and every point of  $t$  belongs to the nucleus of  $\mu(e)$ .*

The proof depends on considerations of minimum intrinsic energy.

LEMMA 2. *Let  $F$  and  $T$  be as in lemma 1 with the proviso, however, that  $F$  be of zero capacity. Let  $V_1(M)$  be a function which is harmonic in  $T$  and bounded below, defined on  $F$  so as to be lower semi-continuous. Then the set  $F + T$  is a domain  $T_1$  and  $V_1(M)$  is super-harmonic in  $T_1$ . The proof of this lemma involves Kellogg's theorem, lemma 1 and the consideration of the functions  $V_1^{(N)}(M)$ , obtained by cutting off the function  $V_1(M)$  by the arbitrary upper bound  $N$ .*

We now apply lemma 2 to our problem, replacing  $F$  by  $s$  (the erstwhile  $\bar{s}$ ), and  $T$  by  $T'$ . We make use of Riesz's theorem on superharmonic functions in order to deduce that in  $T + s$ , which is contained in  $T' + s$ , we may write  $V_1(M) = p(M) + h(M)$ , where  $h(M)$  is harmonic and bounded and  $p(M)$  is a potential of positive mass  $\mu(e)$  on  $s$ . Hence, in  $T + s$ ,

$$V(M) - h(M) = p(M) - \int_{T'} \frac{\rho(P)}{MP} dP, \quad (5)$$

and the right hand member is bounded above.

With the aid again of Kellogg's theorem, this time as applied to univalent subharmonic functions, we can prove

LEMMA 3. *With  $T$  and  $s$  as above, and  $s$  of zero capacity, let  $\mu(e)$  be a positive mass distribution on  $s$ , with  $\mu(s) = 1$ , and  $\nu(e)$  a positive mass dis-*



tribution on  $T$ , with  $\nu(T) = \nu < 1$ . Then the difference of potentials  $\int_s d\mu_p / MP - \int_T d\nu_p / MP$  is unbounded above.

In (5) the density  $\rho(P)$  is summable. Hence the boundedness of its potential in the neighborhood of  $s$  depends merely on the values of  $\rho$  in a neighborhood of  $s$ . Accordingly, with respect to the boundedness of the right-hand member of (5), if  $\mu(e)$  were not identically zero, we could discard temporarily as much of the distribution  $\rho(P)$  as we pleased, outside that neighborhood, and assume that  $\int_T \rho(P) dP < \int_s d\mu_p$ . But then, by lemma 3, the right-hand member of (5) would be unbounded above. Since this is not the case, we must have  $\mu(e)$  identically zero. Hence

$$V(M) - h(M) = - \int_T \frac{\rho(P)}{MP} dP$$

for  $M$  in  $T + s$ . The function  $\rho(P)$ , according to its definition, is regular in the neighborhood of  $T^*$ . We may therefore apply Gauss's theorem to the univalent function  $V(M) - h(M)$ . Finally then

$$\int_{T^*} \frac{dV}{dn} dP = 4\pi \int_T \rho(M) dM = \int_T \nabla^2 V dM,$$

which is the equation (4) to be proved.

<sup>1</sup> Sommerfeld, A., "Über verzweigte Potentiale im Raum," *Proc. London Math. Soc.* 28, 395-429 (1897).

<sup>2</sup> Evans, G. C., "A necessary and sufficient condition of Wiener," *Amer. Math. Monthly*, 54, 151-155 (1947).

<sup>3</sup> If there is more than one branch curve a subdomain  $J$  may be projected on the base space, in order to provide a definition of limit point.

<sup>4</sup> Kellogg, O. D., *Foundations of Potential Theory*, p. 335.

<sup>5</sup> A function is subharmonic in  $T$  if it is subharmonic in every univalent domain contained in  $T$ .

## ON COMPLEXES OVER A RING AND RESTRICTED COHOMOLOGY GROUPS

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The relations between the fundamental group and the homology structure of a space  $S$ , developed recently by several authors,<sup>1</sup> may be formulated in a covering space of  $S$  as relations between the group of covering transformations (automorphisms) and "restricted" cohomology groups,



based upon cochains with certain invariance properties. In this note we establish a general theory of such restricted cohomology groups in a complex with automorphisms,<sup>2</sup> which among other geometric applications contains these relations as a special case. The group ring of the automorphism group, which is a very useful tool, may be replaced throughout by an arbitrary ring, and the whole algebraic formalism is developed for arbitrary "complexes over a ring."

1. *R-complex*. Let  $R$  be a ring with a unit  $e$ . A complex  $\mathcal{C}$  over the ring  $R$ , in short an  $R$ -complex, is a system of  $R$ -modules<sup>3</sup>  $C_n$ ,  $n = -1, 0, 1, 2, \dots$ , which are free for  $n \geq 0$ , and where for each  $n \geq 0$  an  $R$ -homomorphism  $\partial$  of  $C_n$  into  $C_{n-1}$  is given such that  $\partial\partial = 0$ . An element  $a_n \in C_n$  is called an  $n$ -chain;  $\partial a_n$  is its boundary and  $n$  is the dimension of  $a_n$ . Let  $Z_n$  be the kernel of  $\partial$ ,  $n \geq 0$ , and  $Z_{-1} = C_{-1}$ .  $\partial C_{n+1}$  is a subgroup of  $Z_n$ , and as usual  $H_n = Z_n / \partial C_{n+1}$  is called the  $n$ th homology group of  $\mathcal{C}$ ;  $Z_n$  and  $H_n$  are in an obvious way  $R$ -modules.

For a given Abelian group  $J$  (called coefficient group) let  $C^n$  be the group of all homomorphisms of  $C_n$  into  $J$ .  $f^n \in C^n$  is called an  $n$ -cochain; its coboundary  $\delta f^n \in C^{n+1}$  is defined by  $\delta f^n(a_{n+1}) = f^n(\partial a_{n+1})$  for all chains  $a_{n+1}$ .  $\delta$  is a homomorphism of  $C^n$  into  $C^{n+1}$ , with kernel  $Z^n$ . As  $\partial\partial = 0$  implies  $\delta\delta = 0$ ,  $\delta C^{n-1}$  is a subgroup of  $Z^n$  and  $H^n = Z^n / \delta C^{n-1}$ ,  $n \geq 0$ , is the  $n$ th cohomology group of  $\mathcal{C}$  (with coefficient group  $J$ ). When necessary, it will be denoted explicitly by  $H^n(\mathcal{C})$  or  $H^n(\mathcal{C}, J)$ .

2. *Restricted cohomology groups*. Let  $\psi$  be a given group of additive homomorphisms of  $R$  into  $J$ . Instead of considering all cochains  $f^n \in C^n$  we restrict ourselves to  $\psi$ -cochains; that means, to cochains  $f^n$  such that, for each chain  $a_n \in C_n$ ,  $f^n(ra_n)$  considered as function of  $r \in R$  is an element of  $\psi$ . All  $\psi$ -cochains of dimension  $n$  form a subgroup  $C_\psi^n$  of  $C^n$ . The coboundary operator  $\delta$  maps  $C_\psi^n$  into  $C_\psi^{n+1}$ ; for  $\delta f^n(ra_{n+1}) = f^n(\partial ra_{n+1}) = f^n(r\partial a_{n+1})$ , considered as function of  $r \in R$ , is an element of  $\psi$  for each chain  $a_{n+1}$ . Let  $Z_\psi^n$  be the kernel of the homomorphism  $\delta$  of  $C_\psi^n$  into  $C_\psi^{n+1}$ .  $H_\psi^n = Z_\psi^n / \delta C_\psi^{n-1}$ ,  $n \geq 0$ , is the  $n$ th  $\psi$ -cohomology group of  $\mathcal{C}$ .

Examples of homomorphism groups  $\psi$ :

(2.1)  $\psi$  = group of all additive homomorphisms of  $R$  into  $J$ . In this case  $C_\psi^n = C^n$ , and the  $H_\psi^n = H^n$  are the ordinary cohomology groups of  $\mathcal{C}$ .

(2.2) We suppose that  $J$  is an  $R$ -module, and take for  $\psi$  the group of all  $R$ -homomorphisms of  $R$  into  $J$ ;  $f \in \psi$  is given by its value  $f(e)$ , since  $f(r) = r f(e)$  for all  $r \in R$ .  $C_\psi^n$  is the group of all  $R$ -homomorphisms of  $C_n$  into  $J$ ; the  $H_\psi^n$  are called *equivariant* cohomology groups of  $\mathcal{C}$  (cf. §4).

In the following three examples we assume that  $R$  is the *group ring* (with integer coefficients) of a multiplicative group  $G$ . A homomorphism  $f$  of  $R$  into  $J$  is given by its values  $f(x)$  for all  $x \in G$  and may be considered as an arbitrary  $J$ -valued function of  $x \in G$ .

(2.3)  $\psi$  = group of all functions  $f$  such that  $f(x)$  is constant for all  $x \in G$ .

(2.4) We suppose that  $G$  acts as an operator group on  $J$ , and take  $\psi$  to be the group of all functions  $f$  such that  $f(x) = x \cdot f(e)$  for all  $x \in G$ . (2.3) is a special case of (2.4), (2.4) of (2.2).

(2.5) All functions  $f$  which are "almost 0" for all  $x \in G$ —i.e., such that  $f(x) = 0$  except for a finite number of  $x$ —form a group  $\psi$ ; it is different from the trivial one in (2.1) only if  $G$  is infinite.

For any homomorphism  $f$  of  $R$  into  $J$  and any  $s \in R$  we define  $f_s$  by  $f_s(r) = f(rs)$  for all  $r \in R$ . A homomorphism group  $\psi$  will be called *translation invariant*, if it contains with  $f$  all  $f_s$ ,  $s \in R$ . In the  $\psi$ -cochain groups of an  $R$ -complex nothing is changed, when an arbitrary  $\psi$  is replaced by its maximal translation invariant subgroup. All our examples are translation invariant.

3. *The cohomology sequence.* The factor group  $C^n/C_\psi^n$  will be denoted by  $C_r^n$  and called the *residual cochain group* (relative to  $\psi$ ) of dimension  $n$ .  $\delta$  induces a homomorphism of  $C_r^n$  into  $C_r^{n+1}$ , with kernel  $Z_r^n$ , and the groups  $H_r^n = Z_r^n/\delta C_r^{n-1}$ ,  $n \geq 0$ , are the residual cohomology groups of  $\mathcal{C}$  (rel.  $\psi$ ).

The injection  $\iota$  (the identity mapping) of  $C_\psi^n$  into  $C^n$  induces a homomorphism of  $H_\psi^n$  into  $H^n$ . The projection  $\pi$  of  $C^n$  onto its factor group  $C_r^n$  defines a homomorphism of  $H^n$  into  $H_r^n$ . Furthermore, if  $f^n \in C^n$  and  $\pi f^n \in Z_r^n$ , then  $\delta f^n \in C_\psi^{n+1}$ ; and if  $g^n \in C^n$  such that  $g^n$  and  $f^n$  are in the same class mod.  $C_\psi^n$ , then  $\delta g^n - \delta f^n \in \delta C_\psi^n$ ; thus  $\delta$  defines a homomorphism of  $H_r^n$  into  $H_\psi^{n+1}$ . It is easy to see that the homomorphism sequence

$$(3.1) \quad H_\psi^0 \rightarrow \dots \rightarrow H_\psi^n \xrightarrow{\iota} H^n \xrightarrow{\pi} H_r^n \xrightarrow{\delta} H_\psi^{n+1} \rightarrow \dots$$

is exact; i.e., that the image group of any homomorphism of the sequence is the kernel of the following one.

The sequence of cohomology groups (3.1) together with the kernels (or image groups) of the homomorphisms will be called in short the *cohomology sequence* of  $\mathcal{C}$  (rel.  $\psi$ ). A mapping (homomorphism, isomorphism) of a cohomology sequence into another one is a mapping of all groups of the first into the corresponding groups of the second. Two cohomology sequences are isomorphic if all groups of the first are isomorphic to the corresponding groups of the second.

A certain subgroup of  $H_r^n$  will play a rôle later. All cochains  $f^n \in C^n$  such that for each  $z_n \in Z_n$  (not necessarily for each chain  $a_n$ )  $f^n(rz_n)$  considered as function of  $r \in R$  is an element of  $\psi$ , form a subgroup  $\tilde{C}^n$  of  $C^n$ . Obviously  $C_\psi^n \subset \tilde{C}^n$ , and  $\tilde{C}^n/C_\psi^n = \tilde{C}_r^n$  is easily seen to be a subgroup of  $Z_r^n$  and to contain  $\delta \tilde{C}_r^{n-1}$ .  $\tilde{C}_r^n/\delta \tilde{C}_r^{n-1} = \tilde{H}_r^n$  is the subgroup of  $H_r^n$  in question. If  $H_n = 0$ ,  $\tilde{H}_r^n = H_r^n$ .

4. *Complex with automorphisms.*<sup>2</sup> Let  $K$  be a closure finite complex in the sense of combinatorial topology, which admits a group  $G$  of automorphisms without fixed cells; in other words,  $K$  is a covering of a complex  $k$ , and  $G$  the corresponding group of covering transformations. When  $R$  denotes

the group ring of  $G$  with integer coefficients, the groups  $C_n$ ,  $n \geq 0$ , of all finite integer<sup>4</sup>  $n$ -chains of  $K$  are free  $R$ -modules. We define the boundary of a 0-cell to be  $= 1$  and understand by  $C_{-1}$  the additive group of integers, turned into an  $R$ -module by the operation  $x.1 = 1$  for all  $x \in G$ . Then the sequence of all  $C_n$  together with the boundary operator  $\partial$  of  $K$  forms an  $R$ -complex in the sense of §1; we denote it also by  $K$ . Its groups  $H_n$  are the ordinary integer homology groups of  $K$ ,  $n \geq 1$ .  $H_0$  is a subgroup of the usual 0-dimensional homology group of  $K$ ;  $H_{-1} = 0$ .

A cochain  $f^n$  in that  $R$ -complex is given by its values for all cells of  $K$  and may be considered as a  $J$ -valued function of these cells. Let  $\psi$  be a group of  $J$ -valued functions  $f(x)$ ,  $x \in G$  (or a group of additive homomorphisms of  $R$  into  $J$ , cf. §2).  $f^n$  is a  $\psi$ -cochain if for each  $n$ -cell  $c_n$  of  $K$  the function  $f^n(xc_n)$  of  $x \in G$  is an element of  $\psi$ . The set of all cells  $xc^n$ ,  $x \in G$ , for a certain cell  $c_n$ , is called a "transitivity domain" of  $G$ . When the group  $\psi$  is translation invariant, then in order that  $f^n$  is a  $\psi$ -cochain it is sufficient that the property " $f^n(xc_n)$  is an element of  $\psi$ " holds for *one* (arbitrary) cell  $c_n$  of each transitivity domain; we say in short that  $f^n$  is on each transitivity domain an element of  $\psi$  (or "of the type  $\psi$ "). Examples: In the case of the group  $\psi$  defined in (2.3) a  $\psi$ -cochain  $f^n$  is constant on each transitivity domain;  $f^n$  may be considered as an ordinary cochain in  $k$  (for there is a one-one correspondence between the transitivity domains in  $K$  and the cells of  $k$ ), and  $H_\psi^n(K)$  is isomorphic to the ordinary cohomology group  $H^n(k)$ . When  $G$  is an operator group of  $J$  and  $\psi$  given by (2.4), the  $\psi$ -cochains  $f^n$  are such that  $f^n(xc_n) = x \cdot f^n(c_n)$  for all  $x \in G$ ; the "equivariant" groups<sup>5</sup>  $H_\psi^n$  may be interpreted as cohomology groups of  $k$ , where the ordinary incidence numbers are replaced by operators of  $J$  (or, when  $k$  is a geometric complex, as cohomology groups of  $k$  with local coefficients in the sense of Steenrod<sup>6</sup>, for a suitable choice of these local coefficients). When  $\psi$  is defined as in (2.5), a  $\psi$ -cochain is almost 0 (i.e., finite) on each transitivity domain and will be called  $G$ -finite; the resulting  $G$ -finite cohomology groups of  $K$  will be studied in a subsequent note.

5.  $\psi$ -cohomology in an abstract group. For an abstract group  $G$  we denote<sup>7</sup> by  $K_G$  the following abstract closure finite complex: its  $n$ -dimensional cells are the systems  $(x_0, x_1, \dots, x_n)$  of  $n + 1$  elements of  $G$ , and the boundary is defined by  $\partial(x_0, x_1, \dots, x_n) = \sum_{i=0}^n (-1)^i (x_0, \dots, x_{i-1}, x_{i+1}, \dots, x_n)$ . By  $x(x_0, x_1, \dots, x_n) = (xx_0, xx_1, \dots, xx_n)$   $G$  becomes an automorphism group of  $K_G$  without fixed cells. Hence, when a group  $\psi$  of  $J$ -valued functions of  $x \in G$  is given, the  $\psi$ -cohomology groups  $H_\psi^n$  of  $K_G$  are defined; they depend upon  $G$ ,  $J$  and  $\psi$  in a purely algebraic way and may be described by means of  $J$ -valued functions  $f^n(x_0, x_1, \dots, x_n)$  of  $n + 1$  variables  $\in G$  such that  $f^n(xx_0, xx_1, \dots, xx_n)$  considered as function of  $x$  is always an element of  $\psi$ . We denote them by  $\Gamma_\psi^n(G, J)$ .

When  $\psi$  is defined as in (2.3), the  $\Gamma_{\psi}^n(G, J)$  are identical with the cohomology groups of  $G$  studied—by purely algebraic means—by Eilenberg and MacLane<sup>8</sup>.

All homology groups  $H_n$  and cohomology groups  $H^n$  of  $K_G$  are easily seen to be  $= 0$ . From §3 it follows for all  $n \geq 0$  that

$$\tilde{H}_r^n(K_G) = H_r^n(K_G) = H_{\psi}^{n+1}(K_G) = \Gamma_{\psi}^{n+1}(G, J).$$

6. *Chain transformation and homotopy.* We consider again arbitrary  $R$ -complexes (§1). Chain transformation of one  $R$ -complex into another—over the same ring  $R$ —and chain homotopy of such mappings are defined as for ordinary complexes.<sup>9</sup> We add only the condition that all involved homomorphisms  $A$  of chain groups  $C_n$  into other chain groups  $C'_m$ —which are again  $R$ -modules—have to be  $R$ -homomorphisms; consequently (for a given group  $\psi$  of homomorphisms of  $R$  into  $J$ , cf. §2) the dual homomorphisms  $A^*$  of the corresponding cochain group  $C'^m$  into  $C^n$  map  $C'_{\psi}^m$  into  $C_{\psi}^n$  and  $C'^m$  into  $C^n$ , and induce homomorphisms of the different cohomology groups which occur in the cohomology sequence (3.1). It follows easily: When the chain transformation  $W$  of an  $R$ -complex  $\mathcal{C}$  into itself is chain homotopic to the identity mapping of  $\mathcal{C}$ , then the dual mapping  $W^*$  induces the identical isomorphism of the cohomology sequence of  $\mathcal{C}$  and of the groups  $\tilde{H}_r^n$  defined in §3. In the following we shall use a more precise result:

LEMMA (6.1). Let  $W$  be a chain transformation of  $\mathcal{C}$  into itself. If there exist for a certain  $n$  two  $R$ -homomorphisms  $X$  of  $C_{n-1}$  into  $C_n$  and  $Y$  of  $C_n$  into  $C_{n+1}$  such that  $Wa_n - a_n = X\partial a_n + \partial Ya_n$  for all  $a_n \in C_n$ , then  $W^*$  induces the identical isomorphism of all groups and kernels of dimension  $n$  in the cohomology sequence of  $\mathcal{C}$ , and furthermore of  $\tilde{H}_r^{n+1}$  and of  $(H_{\psi}^{n+1})_0$  (i.e., the kernel of the injection  $\iota$  of  $H_{\psi}^{n+1}$  into  $H^{n+1}$ ).

7. *Acyclic  $R$ -complexes.* The  $R$ -complex  $\mathcal{C}$  is said to be acyclic in the dimension  $n$ , if  $H_n = 0$ . The main result of this note concerns  $R$ -complexes which are acyclic in the lowest dimensions  $n < N$ , for a given  $N \geq 0$ . Let  $\mathcal{C}$  be such a complex; straightforward construction yields the two following lemmas (which have nothing to do with the cohomology groups):

LEMMA (7.1). Two chain transformations  $V$  and  $W$  of  $\mathcal{C}$  into itself, which are equal on  $C_{-1}$ , are chain homotopic in all dimensions  $n < N$ ; i.e., there exist for these  $n$   $R$ -homomorphisms  $Y_n$  of  $C_n$  into  $C_{n+1}$ , such that  $Va_n - Wa_n = Y_{n-1}\partial a_n + \partial Y_n a_n$  for all  $n$ -chains  $a_n$ ,  $n < N$ .

LEMMA (7.2). Let  $\mathcal{C}'$  be an  $R$ -complex over the same ring  $R$  as  $\mathcal{C}$ , with  $R$ -modules  $C'_n = 0$  for  $n > N$ . Then an  $R$ -homomorphism of  $C'_{-1}$  into  $C_{-1}$  may be extended to a chain transformation of  $\mathcal{C}'$  into  $\mathcal{C}$ .

Now let  $\mathcal{C}$  and  $\mathcal{C}'$  be  $R$ -complexes over the same ring  $R$ , both acyclic in all dimensions  $n < N$ , and with  $R$ -isomorphic modules  $C_{-1}$  and  $C'_{-1}$ ; we replace all modules  $C_n$  and  $C'_n$  with  $n > N$  by 0. The  $R$ -isomorphism of  $C_{-1}$



onto  $C'_{-1}$  may be extended to a chain transformation  $T$  of  $\mathcal{C}$  into  $\mathcal{C}'$ , and the inverse  $R$ -isomorphism to a chain transformation  $S$  of  $\mathcal{C}'$  into  $\mathcal{C}$ .  $ST$  is a chain transformation of  $\mathcal{C}$  into itself, on  $C_{-1}$  the identity, hence by (7.1) chain homotopic to the identity mapping of  $\mathcal{C}$  in all dimensions  $n < N$ . By (6.1)  $(ST)^* = T^*S^*$  induces the identical isomorphism of the cohomology sequence (3.1) of  $\mathcal{C}$  in all dimensions  $n < N$ , and of  $\tilde{H}_r^N$  and of  $(H_\psi^N)_0$ .  $S^*T^*$  does the same for the corresponding group of  $\mathcal{C}'$ . Therefore,  $S^*$  induces isomorphisms of all involved groups of  $\mathcal{C}$  onto the corresponding ones of  $\mathcal{C}'$ .

**THEOREM (7.3).** *If two  $R$ -complexes over the same ring  $R$  and with  $R$ -isomorphic modules  $C_{-1}$  are acyclic in all dimensions  $< N$ , then their cohomology sequences (rel.  $\psi$ ) are isomorphic in these dimensions, and furthermore their groups  $\tilde{H}_r^N$  and their groups  $(H_\psi^N)_0$  are isomorphic. This holds for any given group  $\psi$  of homomorphisms of  $R$  into the coefficient group  $J$ .*

*Remarks.*—(a) In many cases  $H_n = 0$  for  $n < N$  implies  $H^n = 0$  for  $n < N$ ; e.g., in a complex with automorphisms (§4). Then, in the cohomology sequence (3.1)  $\iota$  and  $\pi$  become 0-mappings for  $n < N$ ,  $\delta$  an isomorphism of  $H_r^{n-1}$  onto  $H_\psi^n$  for  $n < N$  and of  $H_r^{N-1}$  onto  $(H_\psi^N)_0$ . Our result reduces to the fact that the corresponding group  $H_\psi^n$ ,  $n < N$ ,  $(H_\psi^N)_0$  and  $\tilde{H}_r^N$  of the two complexes are isomorphic (or equivalently that their corresponding groups  $\tilde{H}_r^n$ ,  $n \leq N$ , are isomorphic).

(b) By (7.3), in an  $R$ -complex which is acyclic in all dimensions  $< N$ , the cohomology sequence in these dimensions is determined by the abstract structure of  $R$  and  $C_{-1}$ ; we may call it “the cohomology sequence of  $R$  and  $C_{-1}$ ”, for the given  $\psi$ . As for any  $R$  and  $C_{-1}$  there exist  $R$ -complexes which are acyclic in all dimensions<sup>10</sup>, the cohomology sequence (rel.  $\psi$ ) of  $R$  and  $C_{-1}$  is always defined in all dimensions.

8. *Acyclic complexes with automorphisms.* We apply theorem (7.3) to a complex  $K$  with automorphisms (§4), with the automorphism group  $G$ , acyclic in all dimensions  $< N$ , and to the complex  $\bar{K}_G$  (§5). Then, for any coefficient group  $J$  and any group  $\psi$  of  $J$ -valued functions in  $G$ , we obtain for the cohomology groups of  $K$  the isomorphisms

$$\begin{aligned} H_\psi^n &\cong \Gamma_\psi^n(G, J), \quad n < N, \\ (H_\psi^N)_0 &\cong \Gamma_\psi^N(G, J), \\ \tilde{H}_r^N &\cong \Gamma_\psi^{N+1}(G, J). \end{aligned}$$

They give purely algebraic relations between the  $\psi$ -cohomology groups of  $K$  and the automorphism group  $G$ , which contain all previous results<sup>1</sup> of that type as special cases, in particular those concerning relations between the fundamental group of an aspherical space and its cohomology groups (in the ordinary sense, or with local coefficients, corresponding to the equivariant cohomology groups<sup>6</sup> of the universal covering space).

9. The complete statements and proofs together with the discussion and application of special groups  $\psi$  will appear in a forthcoming paper. All results extend in a natural way to the product theory of cochains and cohomology classes. The proof of the topological invariance of the  $\psi$ -cohomology groups of a polytope with automorphisms and the definition of  $\psi$ -cohomology groups for general spaces may be based upon the singular homology theory.

<sup>1</sup> Hopf, H., *Comm. math. helv.*, 17 (1944), pp. 39-79; Eilenberg, S., and MacLane, S., *Ann. of Math.*, 46 (1945), pp. 480-509; Freudenthal, H., *Ann. of Math.*, 47 (1946), pp. 274-316; Eckmann, B., *Comm. math. helv.*, 18 (1946), pp. 232-282; Eilenberg, S., *Trans. Ann. Math. Soc.*, 61 (1947), pp. 378-417.

<sup>2</sup> Cf. for example, G. de Rham, *Comm. math. helv.*, 12 (1939), pp. 191-211; B. Eckmann, ref. 1, pp. 252-254.

<sup>3</sup> An  $R$ -module  $M$  is an Abelian group, additively written, which admits  $R$  as an operator ring, the unit  $e$  of  $R$  being the identity operator.  $M$  is said to be *free*, if it is generated by elements which are linearly independent (over  $R$ ). An  $R$ -homomorphism  $h$  of one  $R$ -module  $M$  into another is a homomorphism such that  $h(ra) = r \cdot h(a)$  for all  $r \in R$  and  $a \in M$ .

<sup>4</sup> The groups  $C_n$  could be defined with other coefficients than integers; then  $R$  would have to be the group ring over these coefficients.

<sup>5</sup> Investigated in detail by Eilenberg (ref. 1).

<sup>6</sup> Steenrod, N. E., *Ann. of Math.*, 44 (1943), pp. 610-627.

<sup>7</sup> Eckmann, B., ref. 1, pp. 257-264.

<sup>8</sup> *Ann. of Math.*, 48 (1947), pp. 51-78 and 326-341.

<sup>9</sup> Cf., for example, S. Eilenberg, *Ann. of Math.*, 45 (1944), pp. 407-447.

<sup>10</sup> See H. Hopf, ref. 1, p. 42.





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## CHROMOSOME STRUCTURAL CHANGES IN *TRADESCANTIA* MICROSPORES PRODUCED BY ABSORBED RADIOPHOSPHORUS

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The utilization of radioisotopes in biological experimentation up to the present time has been mainly concerned with their effectiveness as tracers. There is also the possibility of using the radiations from such substances in producing various cytogenetical changes. Since radioactive isotopes of many elements normally occurring in protoplasm are now available<sup>1</sup> and can be introduced directly into the cells and tissues of organisms, it seemed of particular interest to compare any resulting cytogenetical effects with those produced by various external sources of radiations, about which a considerable amount of information has been accumulated.<sup>2</sup> The present paper deals with preliminary observations of the effects of radiophosphorus absorbed by inflorescences of *Tradescantia* in producing chromosomal structural changes in microspore nuclei.

*Experimental Methods.*—The radiophosphorus used (obtained from the Clinton Laboratories at Oak Ridge) was the separated isotope, phosphorus 32; half-life: 14.3 days,  $\beta$ -radiation: 1.69 Mev. The original solution, whose concentration (activity) when obtained was approximately 600 microcuries ( $\mu$ c.)/ml., was used in four dilutions: A—100  $\mu$ c./ml., B—10  $\mu$ c./ml., C—1  $\mu$ c./ml., D—0.1  $\mu$ c./ml. A clone of *Tradescantia paludosa* was utilized for all cytological observations. The cut ends of ten inflorescences were inserted through holes in lead shields placed over each of four small beakers containing approximately 40 ml. of the dilutions of radiophosphorus used. The lead shields made certain that any effects would be due to the radiophosphorus actually taken up by the plants and not to radiation from the solution. In all the experiments, unless otherwise noted, the inflorescences remained in the solution throughout the period of observation. Further lead shielding was utilized about the inflorescences as protection against emitted  $\beta$ -radiation.

Measurements of the increase in radioactivity due to the uptake of

phosphorus were made with a separate group of inflorescences, utilizing a Lauritsen electroscope. There was a significant increase in activity over the background within about an hour and an increasing rate of activity continued until approximately eight hours after the initiation of the experiment. At this time the rate of increase in activity became approximately constant and remained so for at least four days. Numerous measurements were also made of the activity resulting from phosphorus uptake by individual buds or anthers with a Geiger-Müller counter. In some instances such buds were immediately examined cytologically, making possible a direct comparison of the amount of radioactivity and the aberration frequency.

Aceto-carmin smear preparations of microspores were made at intervals following the start of the experiments and analyzed for chromosome structural changes at the first post-meiotic mitosis.

*Results.*—The results of cytological analyses at three concentrations of radiophosphorus are presented in table 1. With the highest concentration (A—100  $\mu$ c./ml.) satisfactory observations were possible at 24 hours after the treatment started, but the aberration frequency soon became too extensive to permit complete analysis. In a few instances where inflorescences were placed in this concentration of radiophosphorus for a limited period of time and then removed, aberration frequencies were much lower and complete analyses were possible. The types of chromosome structural change found are similar to those induced by other radiations such as x-rays and neutrons.<sup>3, 4, 5</sup> In experiments utilizing external radiations, inflorescences are usually exposed for a brief period to the radiation and cytological examination then made at intervals following treatment. It is found that chromatid aberration types appear at metaphase within a few hours, having been induced in cells in prophase with divided chromosomes at the time of treatment. There is then a transition period with a mixture of types present at about 30 to 40 hours following irradiation after which only chromosome aberration types, produced in cells in the resting stage with effectively unsplit chromosomes, are found. With radiophosphorus treatment chromatid types are also observed initially, and may be quite frequent within 24 hours at higher concentrations—at 100  $\mu$ c./ml. (series A) the frequency of chromatid and isochromatid breaks per 100 cells was 35. Furthermore, table 1 indicates that these types continue to represent the principal component of the aberrations observed up to nine days after the start of treatment with initial phosphorus concentrations of 10  $\mu$ c./ml. or less. The continued presence of chromatid aberration types results from the fact that the developing microspores are exposed to radiations from absorbed radiophosphorus during the entire period of their development, including their passage through prophase up to the metaphase stage at which observations can be made. Chromosome aberration types

appeared after the chromatid types, but were not observed before the fourth day, which is considerably later than was anticipated on the basis of the previous x-ray experiments. This delay in the appearance of break types produced at the resting stage may result from either (a) a retardation in the mitotic cycle in the microspores when subjected to continuous  $\beta$ -radiation from absorbed radiophosphorus, or (b) too low an initial radiation intensity to produce a detectable yield of these types—which in x-ray experiments have been shown to result principally from two breaks produced by independent, but temporally related electron paths.<sup>2</sup> Observations at radiophosphorus concentrations intermediate between those used in series A and B as well as tests of  $\beta$ -radiation from an intense external source should help distinguish between these possibilities.

TABLE 1  
FREQUENCIES OF VARIOUS STRUCTURAL CHANGES PRODUCED IN CHROMOSOMES OF *Tradescantia* MICROSPORE NUCLEI FOLLOWING UPTAKE OF RADIOPHOSPHORUS BY INFLORESCENCES

TIME AFTER START OF EXPERIMENT	NO. OF CELLS EXAMINED	CHROMATID ABERRATIONS PER 100 CELLS		CHROMOSOME ABERRATIONS PER 100 CELLS		
		CHROMATID AND ISO- CHROMATID BREAKS	EXCHANGES	CHROMO- SOME BREAKS	INTER- STITIAL DELETIONS	EXCHANGES (CENTRIC RINGS AND DICENTRICS)
Series B—Initial Concentration of P 32: 100 $\mu$ c./Ml.						
24 hrs.	165	1.2	0.0	0.0	0.0	0.0
48 hrs.	127	13.4	2.4	0.0	0.0	0.0
74 hrs.	130	15.4	4.6	0.0	0.0	0.0
4 days	109	33.0	4.6	2.8	0.0	1.0
5 days	114	28.0	5.3	1.8	0.0	1.0
6 days	108	32.4	6.5	0.0	0.0	1.0
8 days	86	39.5	12.8	1.2	4.7	3.5
9 days	84	37.0	10.8	0.0	1.2	3.6
Series C—Initial Concentration of P 32: 1 $\mu$ c./Ml.						
74 hrs.	146	2.7	0.0	0.0	0.0	0.0
4 days	136	5.9	0.7	0.0	0.0	0.0
9 days	116	11.2	1.7	0.0	1.7	1.7
Series D—Initial Concentration of P 32: 0.1 $\mu$ c./Ml.						
4 days	142	2.8	0.0	0.0	0.0	0.0
9 days	134	2.3	0.0	0.0	0.0	0.0

Table 1 indicates that there is an increase in the frequency of aberrations with time. For series B this increase is approximately linear for chromatid and isochromatid breaks up to four days and is directly correlated with the regular increase in radioactivity of inflorescences as measured with the electroscope. Such a direct relationship is to be expected if these aberrations behave largely as one-hit types with  $\beta$ -radiation as they do with both x-rays and neutrons. Measurements are not yet available to determine

whether the apparent leveling-off in aberration frequency after about four days can be correlated with a corresponding behavior of the curve for radioactivity increase. In a few instances where direct measurements were made of the radioactivity of individual buds or anthers at a given time with a Geiger-Müller counter and the aberration frequency in the same material immediately studied, fairly good correlations of the two measurements were obtained. No systematic attempt has yet been made to determine what fraction of the aberrations is due exclusively to radiation originating within the cells of a given anther or bud, as compared with the irradiation of one bud or inflorescence by  $\beta$ -particles from another.

One striking result of the comparison of aberration frequency types was the relatively high yield of chromatid exchange break types as compared with simple break types. It was early demonstrated in x-ray experiments<sup>3</sup> that exchanges, in contradistinction to simple break types, are aberrations caused by two independent hits (electron paths) and show a time-intensity relationship, such that the yield for a given dose depends on the intensity—decreasing greatly at low intensities. Thus it might be expected that a relatively low yield of exchanges would be obtained with the prolonged radiation at low intensities from radiophosphorus. The relatively high frequency actually observed with  $\beta$ -radiation agrees with the recent analysis of Catcheside, Lea and Thoday<sup>6</sup> which suggests that an appreciable fraction of the exchanges produced at low intensities of x-rays are one-hit types, and further that the time of restitution of broken ends may not be as short as was originally thought.

At increasingly low initial external concentrations of radiophosphorus, decreases in aberration frequency result, though the yields of one-hit types are only roughly proportional to the original external radiophosphorus concentrations, indicating that differences in the degree of phosphorus uptake are probably involved. Even at quite low concentrations (series D), however, where the measured radioactivity of individual buds is of the same order of magnitude as that used in tracer experiments, chromosomal structural changes are still found. Thus it should be realized by investigators utilizing radioisotopes as tracers, especially with biological materials which are maintained in living condition after treatment and used as breeding stocks, that such treatment may result in the production of appreciable numbers of genetic changes. At higher levels of initial radiophosphorus concentration the aberration frequencies, even after relatively short times, may be equivalent to those produced by considerable doses of x-rays. In series A, after only 24 hours, the frequency of chromatid and isochromatid effects (35 per 100 cells) is equivalent to an exposure of the cells to approximately 40 roentgens;<sup>8</sup> in series B, after 9 days, this frequency is the same as that produced by about 50 r. Even though  $\beta$ -radiations are of restricted penetrating power as compared with certain

other radiations, these observations, plus the results of electroscope measurements of inflorescences permitted to take up radiophosphorus from relatively concentrated solutions, indicate that investigators should exercise considerable caution in the use of radioisotopes, especially as regards their close and prolonged exposure to biological materials which have absorbed appreciable quantities of these substances.

*Summary.*—Inflorescences of *Tradescantia paludosa* were permitted to take up radiophosphorus from solutions of various concentrations over a period of nine days. Cytological observations were made at the first post-meiotic (microspore) mitosis at intervals following the initiation of the treatments to determine the types and frequencies of chromosome structural changes which might result from the  $\beta$ -radiation emitted by the radiophosphorus inside the plants. Changes were detected with all of the four concentrations of radiophosphorus used—between the limits of 100  $\mu\text{c./ml.}$  and 0.1  $\mu\text{c./ml.}$  Because of the very high breakage rate at the 100  $\mu\text{c./ml.}$  concentration, aberration frequencies could not be scored after about 48 hours in this series.

The types of aberrations observed were similar to those resulting from the exposure of inflorescences to external radiations such as x-rays and neutrons. There was a fairly regular increase in the frequency of one-hit aberration types with time, which could be correlated with the increase in radioactivity of inflorescences as measured with an electroscope. Chromatid aberrations were observed within 24 hours after the initiation of the treatments and remained the principal type throughout the course of the experiments. Chromatid exchanges occurred with a relatively high frequency as compared with chromatid and isochromatid breaks even at the low radiation intensities involved. Chromosome breaks were not observed until the fourth day. At the highest level of radiophosphorus used (100  $\mu\text{c./ml.}$ ) the frequency of one-hit chromatid aberration types after 24 hours, under the experimental conditions utilized, was equivalent to that produced by about 40 r of x-rays.

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<sup>1</sup> Science, 103, 697-705 (1946).

<sup>2</sup> Lea, D. E., *Actions of Radiations on Living Cells*, Macmillan Company (1947).

<sup>3</sup> Sax, K., *Genetics*, 25, 41-68 (1940).

<sup>4</sup> Giles, N. H., Jr., these PROCEEDINGS, 26, 567-575 (1940).

<sup>5</sup> Catcheside, D. G., *Biological Reviews*, 20, 14-28 (1945).

<sup>6</sup> Catcheside, D. G., Lea, D. E., and Thoday, J. M., *Jour. of Genetics*, 47, 137-149 (1946).



## INTERPOLATION AND UNIQUENESS OF ENTIRE FUNCTIONS

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Let  $\{T_n\}$  be a sequence of linear functionals defined on the class  $K$  of entire functions of exponential type. We raise the following questions:

- (I) Given a sequence of complex numbers  $\{a_n\}$  and a subclass  $C$  of  $K$ , is it possible to find a function  $f$  belonging to  $C$  such that for all  $n$ ,  $T_n(f) = a_n$ ?
- (II) Given a subclass  $C$  of  $K$  and a function  $f$  belonging to  $C$ , what properties of  $f$  can be inferred from the sequence of complex numbers  $\{T_n(f)\}$ ?

- (III) For what subclass  $C$  of  $K$  is it true that if  $f$  belongs to  $C$  and  $T_n(f) = 0$  for all  $n$ , then  $f(z) \equiv 0$ ?

The third is of course a special case of the second, but it is of such special importance that we choose to state it explicitly. A class  $C$  for which it holds will be called a uniqueness class for  $\{T_n\}$ . The first question asks for a solution to the general interpolation problem for the class  $C$  and the functionals  $\{T_n\}$ ; if a function exists having these properties, the sequence  $\{a_n\}$  is said to be admissible for the functionals  $\{T_n\}$  and the class  $C$ . The importance of (III) stems from the fact that if, in (I),  $C$  is a subclass of such a uniqueness class then there can exist at most one such function  $f$  for which  $T_n(f) = a_n$ , while if, in (II),  $C$  is a subclass of a uniqueness class,  $f$  is in fact completely determined by the sequence  $\{T_n(f)\}$  and every property of  $f$  should be inferable from it. This leads naturally to an additional question.

- (IV) When is it possible to expand  $f(z)$  into a series of the form

$$f(z) = \sum u_n(z) T_n(f)?$$

This is the problem of the existence of an interpolation series for the functionals  $\{T_n\}$ , together with the determination of the class of functions for which the series converges. Clearly, any such expansion class is a subclass of the corresponding uniqueness class. In a previous paper,<sup>1</sup> certain aspects of these problems were discussed. Here, the methods used there—due essentially to Pólya<sup>2</sup> and Carlson<sup>3</sup>—are given in a more general form, and are applied to the solution of problem (IV). The treatment is similar to that of Gelfond,<sup>4</sup> but is of much greater power; most of the known results on convergence of Abel, Newton, Stirling, Selberg, as well as other less familiar interpolation series, are obtained at once, and the method extends to discussion of summability. In the present paper, the general method is outlined briefly, and a few of the specific results are stated; detailed proofs will appear in a later paper.

We first seek general representations for functions  $f$  and functionals  $T$ . Some preliminary definitions are necessary. If  $f \in K$ , the class of entire functions of exponential type, so that there exist real numbers  $A$  and  $C$  such that  $|f(z)| \leq A e^{C|z|}$ , then the growth function  $h(\theta, f)$  defined by  $\limsup_{r \rightarrow \infty} r^{-1} \log |f(re^{i\theta})|$  is bounded in absolute value by  $C$ . If  $H(\theta)$  is any function with period  $2\pi$ , then  $K(H(\theta))$  denotes the class of all  $f$  in  $K$  such that  $h(\theta, f) \leq H(\theta)$  for all  $\theta$ ;  $H(\theta)$  may take infinite values. As in previous papers, the notation  $K(a, c)$  is reserved for the class of all  $f$  in  $K$  with  $h(0, f) \leq a$ ,  $h(\pi, f) \leq a$ , and  $h(\pm \frac{\pi}{2}, f) \leq c$ . If  $G$  is any closed set of the complex plane,  $k(\theta, G) = \sup_{w \in G} \Re\{we^{i\theta}\}$  is called the supporting function of  $G$ ; this differs from the usual definition in the sign of  $\theta$ . The function  $k(\theta, G)$  is continuous in  $\theta$ , has period  $2\pi$ , has left and right derivatives everywhere, and is unchanged if  $G$  is replaced by its convex hull. If  $G_1$  and  $G_2$  are point sets, then  $G_1 \cdot G_2$  will be the set of all points of the form  $z'z''$  where  $z' \in G_1$ ,  $z'' \in G_2$ .<sup>b</sup> If  $G_1$  and  $G_2$  are bounded so is  $G_1 \cdot G_2$ ; if  $G_1$  and  $G_2$  are closed and compact, so is  $G_1 \cdot G_2$ ; if  $G_1$  and  $G_2$  are closed bounded convex sets, then  $G_1 \cdot G_2$  is simply connected;  $G_1 \cdot G_2$  is a star set whenever one factor is a star set. If  $f(z) = \sum_0^\infty a_n z^n / n!$ , belonging to  $K$ , then  $\phi(w) = \sum_0^\infty a_n / w^{n+1}$  is usually called the Borel transform of  $f$ . We denote by  $D(f)$  the convex hull of the singularities of  $\phi(w)$ . In our notation, a fundamental theorem due to Pólya<sup>2</sup> states that if  $f$  belongs to  $K$  then  $h(\theta, f) = k(\theta, D(f))$  for all  $\theta$ .

Suppose we consider a function  $\gamma(z) = \sum_0^\infty z^n / n! c_n$  and an associated function  $\gamma^*(z) = \sum_0^\infty c_n z^n / n!$ , both in  $K$ . A modified Borel transform of  $f(z)$  is defined by  $\phi(w) = \sum_0^\infty a_n c_n / w^{n+1}$ .  $\phi(w)$  is regular outside the set  $D(f) \cdot D(\gamma^*)$ , and if  $\Gamma$  is any contour enclosing this set,  $f(z)$  has the representation

$$f(z) = \frac{1}{2\pi i} \int_\Gamma \gamma(zw) \phi(w) dw. \quad (1)$$

Conversely, if  $\gamma \in K$ , if  $G$  is a closed bounded simply connected set, if  $\phi(w)$  is regular outside  $G$  and if  $\Gamma$  is a contour enclosing  $G$  and not passing through the origin, then  $f(z)$ , defined by (1), belongs to  $K$ , and  $h(\theta, f) \leq k(\theta, G \cdot D(f))$ . (The special case for which  $\gamma(z) = e^z = \gamma^*(z)$  was studied by Pólya; it is of interest that if  $D(\gamma) = D(\gamma^*) = 1$ , then  $\gamma(z) = A e^z$ .)

Turning now to functionals, we may obtain a wide variety as follows. Let  $\phi(w)$  be a modified Borel transform of  $f$  with kernel  $\gamma(z)$ , and choose any contour  $\Gamma$  enclosing  $D(f) \cdot D(\gamma^*)$ . Then, for any entire function  $g(w)$ , we define  $T$  by:

$$T(f) = \frac{1}{2\pi i} \int_{\Gamma} g(w) \phi(w) dw. \quad (2)$$

This includes all of the usual cases. In fact, if  $K$  is given the weak topology, any continuous linear functional on  $K$  may be represented in the form (2). When  $T$  is so defined,  $g(w)$  is said to be the generating function of  $T$ .

With this background, we may now study general interpolation series. For simplicity, we confine ourselves to the case where the functionals  $\{T_n\}$  have generating functions  $\{g_n(w)\}$  which are exactly the integral powers of a function  $\zeta(w)$ . This restriction is indeed satisfied for most of the familiar series. If  $\zeta(w)$  is regular and univalent in an open set  $\Omega_w$  of the  $w$ -plane, containing the origin, and if  $\Omega_w$  corresponds to a set  $\Omega_\zeta$  in the  $\zeta$ -plane, then  $w = w(\zeta)$ , regular in  $\Omega_\zeta$ . Since  $\gamma(zw) = \gamma(zw(\zeta))$  is analytic in  $\zeta$  for  $\zeta$  in  $\Omega_\zeta$ ,

$$\gamma(zw) = \sum_0^\infty u_n(z) \zeta^n \quad (3)$$

uniformly convergent in any closed subset of  $\Delta_\zeta$ , the largest open circle  $|\zeta| < R$  contained in  $\Omega_\zeta$ . Let  $\Delta_w$  be the image of  $\Delta_\zeta$ , given by  $|\zeta(w)| < R$ . Then

$$\begin{aligned} \gamma(zw) &= \sum_0^\infty u_n(z) [\zeta(w)]^n \\ &= \sum_0^\infty u_n(z) g_n(w) \end{aligned} \quad (4)$$

is uniformly convergent in any closed subset of  $\Delta_w$ . If  $f$  is such that  $D(f) \cdot D(\gamma^*)$  is interior to  $\Delta_w$ , then we can choose a contour  $\Gamma$  lying in  $\Delta_w$  and enclosing  $D(f) \cdot D(\gamma^*)$ , on which (4) is uniformly convergent. Applying (1) and (2), and integrating termwise, we obtain the convergent interpolation series

$$f(z) = \sum_0^\infty u_n(z) T_n(f). \quad (5)$$

The same procedure will yield results for summability. Let  $E(t) = \sum_0^\infty d_n t^n$  be an entire function, not a polynomial, with  $d_n \geq 0$ . If  $\{S_n\}$  is a sequence of complex numbers with  $|S_n|^{1/n} = o(1)$  then  $\sum_0^\infty S_n d_n t^n$  converges for all  $t$  to a function  $H(t)$ . The limit  $\lim_{t \rightarrow +\infty} H(t)/E(t)$ , if it exists, is the generalized  $E$ -limit of  $\{S_n\}$ . Applied to a power series, the following is true: if  $f(z)$  is regular in a region  $R$  containing the origin, then

$$f(z) = E - \sum_0^{\infty} f^{(n)}(0) z^n/n! \quad (6)$$

where the summability is uniform in any closed compact subset of  $(R' \cdot E')'$ . Here,  $E$  is an open set such that  $\lim_{t \rightarrow \infty} E(zt)/E(t)$  is zero, uniformly in any compact subset of  $E$ .

Returning now to our discussion of interpolation series, we take up  $E$ -summability. Since  $\gamma(zw)$ , as a function of  $\zeta$ , is regular in  $\Omega_1$ , we have

$$\gamma(zw) = E - \sum_0^{\infty} u_n(z) \zeta^n$$

uniformly in any compact subset of  $\Omega_1^* = (\Omega_1' \cdot E')'$ . If  $\Omega_w$  is the image of  $\Omega_1^*$  under  $w = w(\zeta)$ , then

$$\gamma(zw) = E - \sum_0^{\infty} u_n(z) g_n(w)$$

uniformly in any compact subset of  $\Omega_w^*$ . Proceeding as before, we substitute this into (1), and integrate termwise; simplifying by (2), we have

$$f(z) = E - \sum_0^{\infty} u_n(z) T_n(f), \quad (7)$$

holding for all  $f$  such that  $D(f) \cdot D(\gamma^*)$  lies interior to  $\Omega_w^*$ . We summarize these results as follows.

**THEOREM.** Let  $g_n(w) = [\zeta(w)]^n$  be the generating functions of the functionals  $\{T_n\}$ . Then, the formal interpolation series  $\sum_0^{\infty} u_n(z) T_n(f)$  converges to  $f(z)$  for all  $f$  such that  $D(f) \cdot D(\gamma^*)$  lies in  $\Delta_w$ , and is  $E$  summable to  $f(z)$  for all  $f$  such that  $D(f) \cdot D(\gamma^*)$  lies in  $\Omega_w^*$ .

By specialization of the kernel  $\gamma(z)$ , and the function  $\zeta(w)$  and the function  $E(t)$  determining the method of summability, many specific results, both new and old, can be obtained. We indicate briefly only a few of these; more detailed treatments will appear later.

**THEOREM 1.** The Newton expansion  $\sum_0^{\infty} \binom{z}{n} \Delta^n f(0)$  is convergent to  $f(z)$  if  $h(\theta, f) < \theta \sin \theta + \cos \theta \log(2 \cos \theta)$ , is Borel summable to  $f(z)$  if  $f \in K(a, c)$  with  $c < \frac{\pi}{2}$ , and is Mittag-leffler summable to  $f(z)$  if  $f \in K(a, c)$ , with  $c < \pi$ .

**COROLLARY (Carlson<sup>6</sup>).** If  $f \in K(a, c)$ ,  $c < \pi$ , and  $f(n) = 0$  for  $n = 0, 1, 2, \dots$ , then  $f(z) = 0$ .

**THEOREM 2.** The Abel series  $\sum_0^{\infty} z(z-n)^{n-1} f^{(n)}(n)/n!$  is convergent to  $f(z)$  if  $D(f)$  lies in the region  $|we^{1+w}| < 1$ , and is ML summable to  $f(z)$  if

$h(\theta, f) < k(\theta, \Omega_w^*)$  where  $\Omega_w^*$  is the region bounded by the curve  $\rho = (\pi - |\varphi|)/\sin|\varphi|$ .

COROLLARY. If  $f \in K(A)$ ,  $A < 1$ , and  $f^{(n)}(n) = 0$  for  $n = 0, 1, \dots$ , then  $f(z) = 0$ .

THEOREM 3. The Stirling series  $f(0) + \sum_1^\infty \frac{z}{n} \binom{z-1+n/2}{n-1} \Delta^n f\left(-\frac{n}{2}\right)$  is convergent to  $f(z)$  if  $D(f)$  lies in the region  $|\sinh(w/2)| < 1$ , is Borel summable to  $f(z)$  if  $f \in K(\pi/\sqrt{2})$ , and is ML summable if  $f \in K(a, c)$  with  $c < \pi$ .

THEOREM 4. The series  $f(0) + \sum_1^\infty \frac{z}{n} \binom{z-n-1}{n-1} \Delta^n f(n)$  is convergent to  $f(z)$  if  $f \in K(A)$ , with  $A < \log(1 + \sqrt{2})/2$  and is ML summable if  $h(\theta, f) < (-\theta) \sin \theta + \cos \theta \log(-2 \cos \theta)$  for  $\frac{\pi}{2} \leq |\theta| \leq \pi$ .

THEOREM 5. If  $D(f)$  lies in the region bounded by the curve  $u = \log \sin \beta v - \log \sin(\beta + 1)v$ , for  $\beta > 0$ , then

$$f(z) = ML - \left\{ f(0) + \sum_1^\infty \frac{z}{n} \binom{z-\beta n-1}{n-1} \Delta^n f(\beta n) \right\}.$$

Several of the above theorems can be strengthened considerably, but for simplicity of statement only the weaker forms have been given. In particular, the uniqueness theorems obtained by Gelfond<sup>4</sup> by methods of conformal mapping become immediate corollaries of theorems on the summability of the corresponding interpolation series. It is also possible to treat series arising from sequences of functionals  $\{T_n\}$  whose generating functions are not of the form  $[f(w)]^n$ . The Lidstone series<sup>7</sup> is a simple example of such a series.

THEOREM 6. The Lidstone series  $\sum_0^\infty \Lambda_n(z) f^{(2n)}(1) + \sum_0^\infty \Lambda_n(1-z) f^{(2n)}(0)$  are convergent to  $f(z)$  if  $f \in K(A)$ ,  $A < \pi$ , are Borel summable to  $f(z)$  if  $f \in K(a, c)$ ,  $c < \pi$ , and are ML summable to  $f(z)$  if  $D(f)$  does not contain any points of the vertical lines  $u = 0$ ,  $|v| \leq \pi$ .

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<sup>1</sup> Buck, R. C., *Duke Journal*, 13, 541-559 (1946).

<sup>2</sup> Pólya, G., *Math. Zeit.*, 29, 549-640 (1929).

<sup>3</sup> Carlson, F., *Sur une classe de séries de Taylor*, Thesis, Uppsala.

<sup>4</sup> Gelfond, A., *Math. Sbornik*, 4 (new series), 115-147 (1938).

<sup>5</sup> Not to be confused with  $G_1 \cap G_2$  which denotes the intersection of the sets  $G_1$  and  $G_2$ . In general,  $G'$  will denote the complement of  $G$ .

<sup>6</sup> Hardy, G. H., *Acta Math.*, 42, 327-339 (1920).

<sup>7</sup> Boas, R. P., Jr., *Duke Journal*, 10, 239-245 (1943).

## ON A THEOREM OF BANACH

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Consider a trigonometric series

$$\sum_{n=1}^{\infty} (a_n \cos nx + b_n \sin nx) \quad (1)$$

and any sequence of positive integers  $n_1 < n_2 < \dots$  satisfying an inequality

$$n_{k+1}/n_k > q > 1 \quad (k = 1, 2, \dots)$$

where  $q$  is independent of  $k$ . Such sequences  $\{n_k\}$  are often called *lacunary*. The following two results are due to Banach:<sup>1</sup>

(i) *Given any sequence  $\alpha_1, \beta_1, \alpha_2, \beta_2, \dots$  of real numbers tending to 0, there is always a Fourier-Lebesgue series (1) such that*

$$a_{n_k} = \alpha_k, \quad b_{n_k} = \beta_k \quad \text{for } k = 1, 2, \dots \quad (2)$$

(ii) *Given any sequence of real numbers  $\alpha_1, \beta_1, \alpha_2, \beta_2, \dots$  such that  $\sum (\alpha_k^2 + \beta_k^2) < \infty$ , there is always a series (1) which is the Fourier series of a continuous function  $f(x)$  and which satisfies (2).*

While there exist simple proofs of (i), based on the considering of the (F. Riesz') products<sup>2</sup>

$$\prod_{k=1}^{\nu} (1 + \alpha_k \cos n_k x + \beta_k \sin n_k x) \quad (3)$$

the existing proofs of (ii) are long and complicated. In this note we shall show that a simple modification of the product (3) immediately leads to a proof of (ii).

First of all, we observe that it is enough to prove a slightly weaker variant of (ii), with the condition of continuity of  $f$  there replaced by that of boundedness. For let  $\epsilon_1, \epsilon_2, \dots$  be any positive and convex sequence of numbers tending to 0, so slowly, however, that  $\sum (\alpha_k^2 + \beta_k^2) \epsilon_{n_k}^{-2}$  still converges. Suppose that (ii) has been established in the weaker form just mentioned. Then there is a series (1) which is the Fourier series of a bounded function  $f$  and such that

$$a_{n_k} = \alpha_k \epsilon_{n_k}^{-1}, \quad b_{n_k} = \beta_k \epsilon_{n_k}^{-1}. \quad (4)$$

It is known however<sup>3</sup> that if we multiply the  $n$ th term of the Fourier series



of a bounded function by  $\epsilon_n$  ( $n = 1, 2, \dots$ ) the resulting series is the Fourier series of a continuous function. Thus  $\sum (a_n \cos nx + b_n \sin nx) \epsilon_n$  is the Fourier series of a continuous function, and, by (4), the coefficients of the latter series at the places  $n_k$  are  $\alpha_k, \beta_k$ .

Passing to the proof of (ii), with  $f$  merely bounded, let us consider the products

$$P_\nu = i^{-1} \prod_{k=1}^{\nu} \{1 + iA_k(x)\} \quad (\nu = 1, 2, \dots) \quad (5)$$

where  $i = \sqrt{-1}$  and  $A_k(x) = \alpha_k \cos n_k x + \beta_k \sin n_k x$ . These products are very similar to (3), so that certain well-known properties of the latter<sup>4</sup> remain obviously valid. Let us list them:

(a) For  $q \geq 3$ , if we multiply  $P_\nu$  out and replace the products of cosines and sines by linear combinations of cosines and sines, all the terms we get are distinct. In particular, the terms of  $P_\nu$  with indices  $n_k$  ( $k = 1, 2, \dots, \nu$ ) are  $\alpha_k \cos n_k x + \beta_k \sin n_k x$ .

(b) For  $q \geq 3$ ,  $P_\nu$  is a partial sum of  $P_{\nu+1}$ .

Hence assuming that  $q \geq 3$  and making  $\nu \rightarrow \infty$  in (5) we obtain formally a trigonometric series (1) satisfying (2). The  $P_\nu(x)$  are uniformly bounded, since

$$|P_\nu| \leq \left\{ \prod_{k=1}^{\nu} (1 + \alpha_k^2 + \beta_k^2) \right\}^{1/2} \leq \left\{ \prod_{k=1}^{\infty} (1 + \alpha_k^2 + \beta_k^2) \right\}^{1/2} < \infty.$$

It immediately follows that the so-obtained series is the Fourier series of a bounded function  $f(x)$ , and (ii) is proved for  $q \geq 3$ . Our function  $f$  is in general complex valued, but obviously the real part of  $f(x)$  also satisfies the required conditions.

To get rid of the condition  $q \geq 3$  we proceed as in the proof of (i). As there,<sup>5</sup> we show that

(c) Let  $\epsilon$  be a fixed but arbitrarily small positive number. If  $q$  is large enough,  $q \geq q_0(\epsilon)$ , all the indices  $\mu$  actually occurring in the trigonometric polynomial  $P_\nu$ —and so also of the series (1) which is the formal limit of the  $P_\nu$ —are situated in the intervals  $(n_k(1 - \epsilon), n_k(1 + \epsilon))$ .

Let now  $q$  be any number greater than 1. Let  $r$  be any positive integer, and let us split the sequence  $\{n_k\}$  into  $r$  sequences

$$n_k^{(s)} = n_{s+kr} \quad (s = 1, 2, \dots, r; \quad k = 0, 1, 2, \dots)$$

Obviously  $n_{k+1}^{(s)} / n_k^{(s)} > q^r$  can be as large as we please, provided  $r$  is large enough. In particular, we require that  $q^r \geq 3$ . We can construct a series (1), which we shall denote by  $T_s$  ( $s = 1, 2, \dots, r$ ), such that  $T_s$  is the Fourier series of a bounded function and that  $T_s$  has the prescribed coefficients ( $= \alpha_{s+kr}, \beta_{s+kr}$ ) at the places  $n_{s+kr}$ . In addition, if  $r$  is large enough,  $T_s$  will only have indices in the intervals  $(n_{s+kr}(1 - \epsilon), n_{s+kr}(1 + \epsilon))$ .

$(1 + \epsilon)$ ). Hence, if  $r$  is large enough, the series  $T_1, T_2, \dots, T_r$  do not overlap, and the series  $T = T_1 + T_2 + \dots + T_r$  has the required properties.

<sup>1</sup> For the literature, see Zygmund, A., *Trigonometrical Series* (quoted hereafter *TS*), p. 215.

<sup>2</sup> See *TS*, p. 220.

<sup>3</sup> *TS*, pp. 104, 105.

<sup>4</sup> *TS*, p. 139.

<sup>5</sup> *TS*, p. 139.

## THE INVERSION OF A GENERALIZED LAPLACE TRANSFORM

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In these PROCEEDINGS<sup>1</sup> the author showed how the convolution transform

$$f(x) = \int_{-\infty}^{\infty} G(x-t)\phi(t)dt \quad (1)$$

can be inverted by a linear differential operator of infinite order. The kernel  $G(x)$  was any function of the form

$$G(x) = \frac{1}{2\pi i} \int_{c-i\infty}^{c+i\infty} \frac{e^{st}}{E(s)} ds \quad 0 < c < a_1, \quad (2)$$

where

$$E(s) = s \prod_{k=1}^{\infty} \left( 1 - \frac{s^2}{a_k^2} \right)$$

$$0 < a_1 < a_2 < \dots$$

$$\sum_{k=1}^{\infty} \frac{1}{a_k^2} < \infty.$$

The inversion of the Stieltjes transform was included as the special case  $a_k = k$ ,  $E(s) = \pi^{-1} \sin \pi s$ . The Laplace transform was not included in the general theory since, as we shall see, it requires the introduction of a function  $E(s)$  whose roots are not symmetrically situated with respect to the origin.

Let us make an exponential change of variable in the Laplace transform

$$F(s) = \int_0^{\infty} e^{-st} \Phi(t) dt. \quad (3)$$

Then equation (3) takes the form (1) with

$$f(x) = F(e^{-x}), \quad G(x) = e^{-e^{-x}}, \quad \phi(x) = \Phi(e^x)e^x.$$

Since

$$\Gamma(s) = \int_0^\infty e^{-u} u^{s-1} du = \int_{-\infty}^\infty e^{-u} e^{-s^{-1}u} du,$$

we have by the classical inversion of the bilateral Laplace transform that

$$e^{-s^{-1}x} = \frac{1}{2\pi i} \int_{c-i\infty}^{c+i\infty} e^{sx} \Gamma(s) ds \quad 0 < c < \infty.$$

This equation has the form (2) with  $E(s)$  replaced by  $1/\Gamma(s)$ , an entire function with its roots at the points  $0, -1, -2, \dots$ . With this example before us, let us now proceed with the more general theory.

Set

$$E(s) = s^p \prod_{k=1}^\infty \left(1 - \frac{s}{a_k}\right) e^{s/a_k}, \quad (4)$$

where  $p$  is a non-negative integer and the  $a_k$  are real constants such that

$$0 < |a_1| \leq |a_2| \leq \dots \quad (5)$$

$$\sum_{k=1}^\infty \frac{1}{a_k^2} < \infty. \quad (6)$$

Under these conditions it can be proved that  $1/E(s)$  is a bilateral transform of a function  $G(x)$  which we take to be the kernel of the transform (1). Our principal result is that this transform can be inverted by the linear differential operator of infinite order  $E(D)$ . That is,

$$E(D)f(x) = \phi(x)$$

$$\begin{aligned} &= \lim_{n \rightarrow \infty} D^p \prod_{k=1}^n \left(1 - \frac{D}{a_k}\right) e^{D/a_k} f(x) \\ &= \lim_{n \rightarrow \infty} D^p \prod_{k=1}^n \left(1 - \frac{D}{a_k}\right) f(x + s_n), \end{aligned}$$

where  $D$  stands for differentiation with respect to  $x$  and

$$s_n = \sum_{k=1}^n \frac{1}{a_k}.$$

We state the result in the form of a theorem.

**THEOREM.** *If  $\phi(x)$  is continuous and absolutely integrable on  $(-\infty, \infty)$ , if  $E(s)$  is defined by equations (4) (5) (6),  $G(x)$  by (2) and  $f(x)$  by (1), then*

$$E(D)f(x) = \phi(x). \quad (7)$$

Let us now show that the Post-Widder<sup>2</sup> inversion formula

$$\lim_{n \rightarrow \infty} \frac{(-1)^n}{n!} \left(\frac{n}{t}\right)^{n+1} f^{(n)}\left(\frac{n}{t}\right) = \Phi(t) \quad (8)$$

for the Laplace transform (3) is included in the above theorem. Let us use the Euler<sup>3</sup> form of the infinite product expansion of  $\Gamma(s)$ :

$$\begin{aligned} \frac{1}{\Gamma(s)} &= s \prod_{k=1}^{\infty} \left(1 + \frac{s}{k}\right) \left(1 + \frac{1}{k}\right)^{-s} \\ &= \lim_{n \rightarrow \infty} n^{-s} s \prod_{k=1}^n \left(1 + \frac{s}{k}\right). \end{aligned}$$

Then equation (7) becomes

$$\lim_{n \rightarrow \infty} D \prod_{k=1}^n \left(1 + \frac{D}{k}\right) f(x - \log n). \quad (9)$$

But if we set  $e^{-x} = t$  and observe the relation  $D_x = -tD_t$ , equation (9) becomes

$$\lim_{n \rightarrow \infty} -tD_t \prod_{k=1}^n \left(1 - \frac{tD_t}{k}\right) F(nt) = \Phi\left(\frac{1}{t}\right) \frac{1}{t}.$$

Simple computation shows that this equation is equivalent to

$$\lim_{n \rightarrow \infty} \frac{(-nt)^{n+1}}{n!} F^{(n+1)}(nt) = \Phi\left(\frac{1}{t}\right) \frac{1}{t}.$$

If  $t$  is replaced by its reciprocal this equation is seen to be the same as (8).

It is important to note the equality signs in the relations (5). Thus multiple roots are permitted in the function  $E(s)$ . As a consequence all the successive iterated Laplace transforms are subsumed under the present theory. They are inverted by use of equation (7) where  $E(s)$  has multiple roots (in the case of iterates beyond the first). That is, suitable modifications of the Post-Widder operator will be effective.

<sup>1</sup> Widder, D. V., "Green's Functions for Linear Differential Systems of Infinite Order," *Proc. Nat. Acad. Sci.*, **33**, 31-34 (1947).

<sup>2</sup> Widder, D. V., "The Laplace Transform," Princeton (1946), p. 288.

<sup>3</sup> Whittaker, E. T., and Watson, G. N., "A Course of Modern Analysis," Cambridge (1943), p. 237.

# THE IODINE METABOLISM OF *DROSOPHILA GIBBEROSA* STUDIED BY MEANS OF RADIOIODINE $I^{131}$ \*

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*Introductory.*—The very little knowledge available concerning the iodine metabolism of invertebrates is confined to the occurrence of accumulation of the element in sponges and gorgonians.<sup>1</sup> It therefore seemed desirable to attack the problem by using  $I^{131}$  in some other organism. Because *Drosophila gibberosa* is a holometabolous insect, it seemed that valuable information might be obtained which would not only be of interest from the standpoint of iodine metabolism of this fly but would also provide a convenient basis for comparative work on the iodine metabolism of some other insect or arthropod.

*D. gibberosa*, a giant black Mexican species, was sent to this laboratory through the courtesy of Dr. T. Dobzhansky. The size of *D. gibberosa* as compared with the smaller *D. melanogaster* is of considerable advantage especially in carrying out the technical side of this problem.

*Material and Methods.*—Radioactive carrier-free iodide was made available to both third instar larvae and adults by stirring 0.2 ml. of a solution of the isotope into approximately 8 ml. of liquid corn meal, molasses and dried Brewer's yeast mixture which had been placed in a creamer bottle. The largest quantities of  $I^{131}$  used were 0.2 mc.

TABLE 1

Jaws and Anterior Fore Gut Chitin.....	34.3%
Larval Skin.....	29.8
Tracheal Trunks and Posterior Spiracles.....	20.1
Imaginal Discs.....	3.1
Fat Body.....	2.7
Ring Gland.....	3.6
Fore and Mid Gut.....	0.0
Hind Gut.....	2.7
Salivary Glands.....	0.0
Malpighian Tubes.....	2.7
Cerebral Ganglia and Nerve Cord.....	0.94

Both larvae and adults were allowed to feed on the radioactive food for varying periods of time, after which dissections were made and measurements of activity taken with a Geiger counter. A second more sensitive method for detecting radioactivity was employed with the larvae. This involved making radioautographs by mounting alternate paraffin ribbon sections of the larvae on dental x-ray film, exposing for periods of 3–5 days

and developing after removal of the paraffin. The other alternate series was mounted on glass slides for iron hematoxylin staining.

*Larval Distribution.*—Table 1 indicates the distribution of  $I^{131}$  in a third instar larva after feeding on radioactive medium for 72 hours. The percentages in this instance were calculated on the basis of the sum of the individual Geiger counts from each dissection in this experiment, disregarding the loss of activity due to dissection of the larva in either alcohol or insect Ringer. No counts are available in this instance to indicate the amount of activity left in the dissection fluid, but as the tissues were dissected in great detail, the data form a convenient introduction to further discussion.

These results indicate clearly that the iodine is concentrated in the skeletal parts of the larva. It is worth noting that no concentration of  $I^{131}$  appeared in any of the larval endocrine structures.

Series of experiments were carried out in which third instar larvae fed on the radioactive food for six different time intervals varying from  $17\frac{1}{2}$ –96 hours. After removal from the bottles, and following a washing in insect Ringer solution in order to remove any excess food adhering to the surface, Geiger counts were taken on whole larvae which had been teased apart. These larvae were then removed from the Geiger counter, dissected completely and counts taken on the individual tissues dissected. The mean per cent of activity recovered from eleven such series of counts on dissected larvae was 125.2% of that of counts on the teased larvae. The variation in excess of 100% is undoubtedly due to two factors. In the first place, it is impossible to obtain completely accurate counts on the teased entire larvae due to the thickness of the incompletely separated tissues which must absorb some  $\beta$ -radiation. In the second place, it is extremely difficult to duplicate precisely the identical geometrical counter relationships for each individual dissection made.

TABLE 2

## MEAN PERCENTAGES OF RADIOACTIVITY

Larval Skin.....	72.7%
Jaws, Anterior and Posterior Spiracles.....	12.7
Tracheal Trunks.....	2.8
Residue.....	11.6

The teased larvae were separated into the following dissected parts: larval skin; tracheal trunks; jaws, anterior and posterior spiracles; and residue which included all remaining tissues and body fluids of the organism. Table 2 indicates the mean of the percentages of activity found in these parts. The sum of the counts for the four individual dissections was used as 100% in calculating the percentages. The age of the larva apparently is not a factor in determining the percentage of radioactivity in the larval skin.



The radioautographs likewise show clearly the concentration of radioactivity which is present in the skeletal structures of the larva. Figure 1 is a longitudinal section, not including the extreme anterior end, cut across the dorsal surface of a third instar larva. A radioautograph, figure 2, made from an alternate section of the same third instar larva as seen in figure 1, indicates that the concentration of greatest radioactivity was in the larval skin and tracheal trunks. The cross section of a third instar larva seen in figure 3 is cut through the hind gut and anal regions. Attention should be directed to the very large hypodermal cells located ventrally and extending only to the midlateral regions of the larva. Figure 4 shows that the radioactivity was extremely high in this region. It seems likely that these large cells have in some way been active in concentrating the radioiodine in the cuticle under which they lie.

Skins of the larvae which were highly radioactive were treated for 48 hours with hot 5% KOH (65°C.). At the end of this period, Geiger counts indicated that virtually all of the radioactive material had been removed, none being left in the chitin fractions which remained after the KOH treatment. This suggests that the radioiodine is present in a protein portion of the skin rather than in the chitin.<sup>2, 3</sup>

Inasmuch as the larvae were crawling about in the radioactive food, the question arises as to whether the iodine in the exoskeleton may represent purely physical uptake by the external surface of the animal. When fresh-cleaned skins of third instar larvae were placed in a radioactive iodide solution for a period as long as six days, there was no indication from Geiger counts that any  $I^{131}$  had been removed from the medium. If physical uptake were responsible for the radioactivity found in the intact larval skins, the skins immersed in iodide solution would presumably have given some

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#### Explanation of Figures

Abbreviations: A, Anus; FB, Fat Body; HG Hind Gut; LHC, Large Hypodermal Cells; LS, Larval Skin (includes both hypodermal cells and underlying cuticle); M, Muscle; TT, Tracheal Trunk.

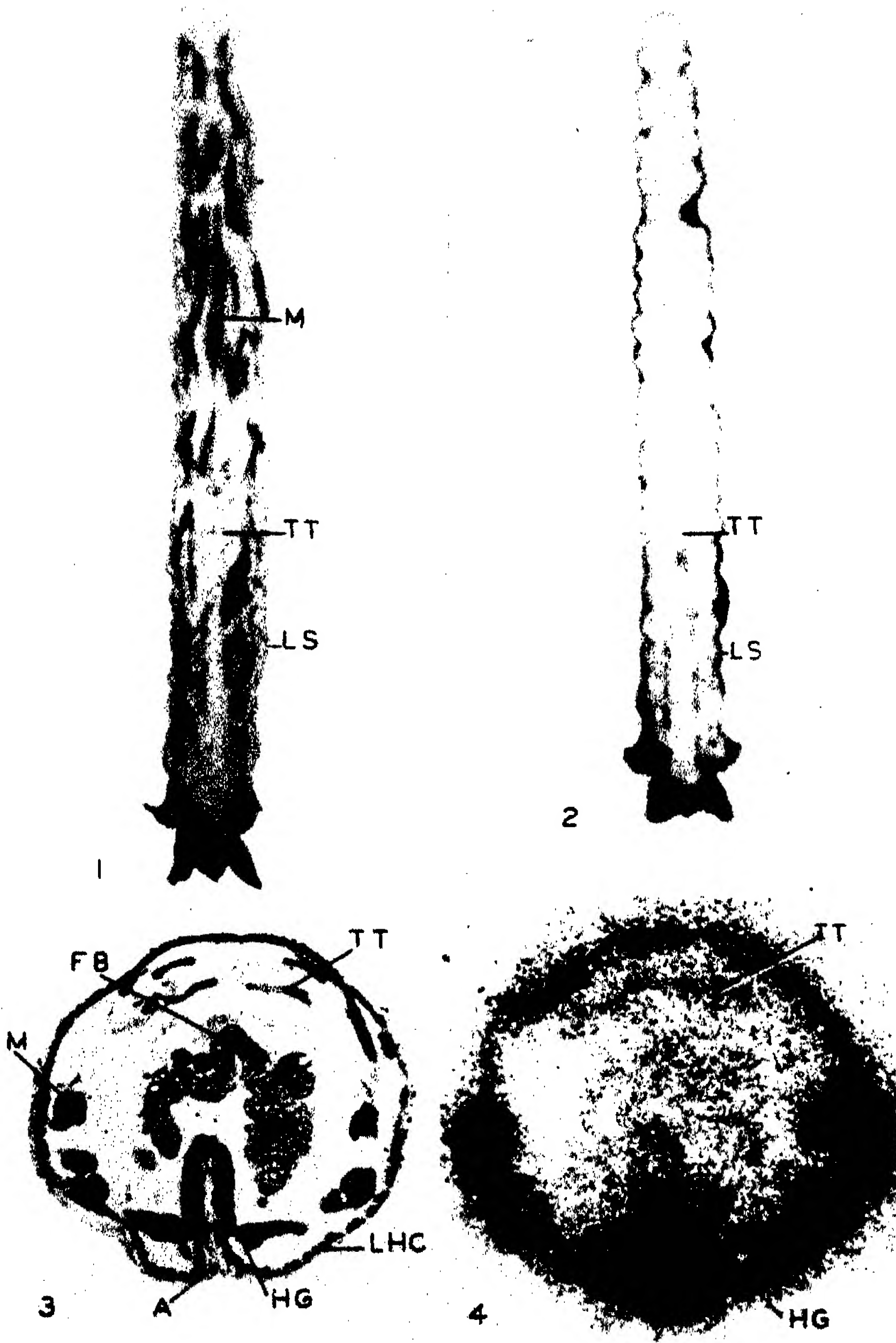
Longitudinal sections,  $\times 23$ ; cross sections,  $\times 96$ .

Figure 1. Longitudinal section of a third instar larva stained with iron hematoxylin.

Figure 2. Radioautograph made from the longitudinal section adjacent to that in figure 1. The darkened areas represent reduced regions of the x-ray film which coincide with the accumulation of  $I^{131}$  in the tissues sectioned.

Figure 3. Transverse section through the anus and hind gut region of a third instar larva stained with iron hematoxylin. Note the general topography for interpretation of figure 4.

Figure 4. Radioautograph made from the cross section adjacent to that in figure 3. The marked  $I^{131}$  accumulation located ventrally and extending midlaterally in the section coincides with the region of the large hypodermal cells and adjacent larval cuticle figure 3.



(For explanation of figures see opposite page).



indication of these processes. If second instar larvae which have been feeding on  $I^{131}$  are removed from the food just prior to molting, and are washed and then allowed to molt after crawling on moist filter paper, Geiger counts of the young third instar larvae indicate a high degree of radioactivity. There is no possible way for these larvae to have acquired this amount of radioactivity except by first feeding and then depositing the  $I^{131}$  in the skeletal components. It is therefore legitimate to conclude that the deposition of iodine in the protein of the skeleton represents a genuine metabolic treatment by the organism of iodine taken up in the food.

*Pupal Period.*—Larvae which had fed on radioactive food were allowed to pupate in the bottles. The pupae (ages 24 hours and 96 hours) were then removed, washed and dissected free of the pupa cases. Geiger counts were taken on samples, each of which consisted of a tanned puparium and its pupa. These were then separated completely and individual counts made on the puparium and pupa. The mean of the percentage of activity recovered from the completely separated portions was 114.3%. The reasons for this mean being in excess of 100% have already been stated in the case of the larvae. When the sum of the counts for the two individual dissected structures was used as 100%, the mean percentage of radioactivity in the puparium was 85.0%, in the pupa, 14.9%. Inasmuch as the pupal skin remained intact, it seems likely that most of the 14.9% of activity recorded for the pupa was located in the pupal skin, which is very hard to separate, rather than in the developing imago or the histolysed larval tissues. In contrast to the larval skin, the tanned puparium can incorporate  $I^{131}$  if placed in an iodide solution.

*Larval Acquisition.—Adult Emergence.* Pupae which had formed from larvae having fed on  $I^{131}$  were removed from the food before emergence of the imagos, washed and placed on moist filter paper in a vial. After emergence of the adults, complete dissections were made and counts taken on the dissected tissues. In no instance was there any significant radioactivity. It would appear that the larva and subsequently the pupa use the molted exoskeletons as a depot for any iodine that may enter. As far as the pupa and imago are concerned, the element can have little if any physiological significance. It should be noted that the quantity of iodine actually administered in a carrier-free  $I^{131}$  solution is excessively small and therefore likely to give a true picture of the movement of minute natural concentrations of stable iodine isotopes in the food.

*Adult Acquisition.*—Pupae, formed from larvae which had never fed on  $I^{131}$ , were buried in the radioactive food and imagos allowed to emerge, thereby assuring the adults of radioactive food only. When counts were taken on dissections of tissues of the adults after 4–5 days' feeding, the highest percentages of radioactivity were located again in the exoskeleton.

Table 3 includes all dissections which indicated 10% or more of the total activity calculated on the basis of the sum of the individual counts being equal to 100%.

TABLE 3

Thoracic Exoskeleton.....	31.6%
Legs.....	18.1
Abdominal Exoskeleton.....	14.0
Head (-the Eyes).....	10.3

Additional data indicate that the iodine is probably in association with a protein complex in the adult exoskeleton as is the case in the larva. When portions of radioactive adult exoskeletons were treated with hot 5% KOH, the activity was lost. The chitin which remained after the treatment was free of any radioactivity.

*Geochemical and Comparative Biochemical Significance.*—It seems probable that the capacity to link iodine to a protein complex in the exoskeleton is characteristic not only of *D. gibberosa* but is typical of other insects and possibly other arthropods as well. If one considers the large amount of oceanic crustacean plankton, and if the organisms comprising this population also can form iodinated scleroproteins, an extremely large amount of the iodine present in the ocean might well be bound by their exoskeletons and be continually sedimented as exuviae.

It is known that some sponges and gorgonians accumulate large amounts of bromine and iodine in the form of dibromotyrosine and diiodotyrosine.<sup>1</sup> In view of the results obtained on *D. gibberosa*, it is reasonable to suppose that the concentration of these two halogens in the skeletons of such organisms is an inevitable result of the formation of scleroproteins in sea water containing iodine and bromine. If tyrosine is an important component amino acid of the protein complex in the larval skin of *D. gibberosa*, it should be possible to demonstrate traces of diiodotyrosine in the exoskeleton of this insect as well as in the lower invertebrates.

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## THE RELATIONSHIP OF GROWTH HORMONES AND FRUIT DEVELOPMENT

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Fruit development is normally the result of pollination and fertilization although numerous instances of parthenocarpy and parthenogenesis have been reported in the literature. It has been shown (Muir<sup>11</sup>) that considerable quantities of diffusible growth hormones are released in the style and ovary of *Nicotiana tabacum* following pollination and fertilization. The investigations of Fitting,<sup>5</sup> Laibach,<sup>10</sup> Thimann<sup>14</sup> and others have indicated that pollen is a relatively rich source of growth hormones. With this in mind Gustafson<sup>7</sup> postulated that the growth hormones from the pollen grains and pollen tubes initiate the early stages of fruit development and after fertilization the developing embryo provides additional growth hormones to other portions of the ovary for its development. Van Overbeek, Conklin and Blakeslee<sup>17</sup> have stated that the pollen of an ordinary pollination does not contain sufficient auxin to be the sole cause of fruit development. They suggest that the substance from the pollen which initiates enlargement of the ovary and ovule may be a prosthetic group which properly combined in the ovary forms an enzyme which activates the auxin precursor. The verification of either hypothesis requires the examination of the possible sources of the growth hormones involved in fruit development on a quantitative basis.

Experiments were performed to determine (1) the amount of growth hormones in the pollen grain which could be transported through the pollen tube to the ovary; (2) the production of growth hormones during the development of the pollen tube; (3) the amount of growth hormones in the ovary before fertilization; (4) the production of growth hormones in ovary tissues by the action of pollen extracts.

*Bioassay of Growth Hormones.*—Determinations of growth hormone concentrations were made according to the modification suggested by Skoog<sup>12</sup> of the standard *Avena* test as described by Went and Thimann.<sup>18</sup>



Curvatures are expressed as the arithmetical mean of the test row of plants with the standard error of the mean and are recorded for the same agar dilution in each instance. Direct comparisons of curvatures are valid in any given experiment. To correct for differences in the sensitivity of the test plants in different experiments the curvatures are translated into the number of micrograms of indoleacetic acid required to produce an equivalent amount of curvature of the *Avena* coleoptile as is produced by the growth hormones obtained from the test material. The calculation employs the following formula:

$$\frac{\text{Micrograms Indoleacetic Acid}}{\text{Milligram of Material}} = \frac{V_1 \times C_1 \times 2.5 \times 10^{-4}}{V_2 \times C_2 \times W}$$

$V_1$  = volume of agar dilution of the extract

$V_2$  = volume of agar block applied to each coleoptile = 0.0125 ml.

$C_1$  = average curvature of coleoptile induced by the extract

$C_2$  = average curvature of coleoptile induced by  $2.5 \times 10^{-4}$  micrograms of indoleacetic acid

$W$  = fresh weight of material extracted in milligrams

*Growth Hormones in Pollen Grains and Pollen Tubes.*—A weighed quantity of viable pollen was ground with powdered glass and a few drops of a 10-ml. volume of 0.1 *N* HCl until examination under the microscope revealed no whole grains. The remaining acid was used to transfer the mixture quantitatively to a separatory funnel. The pH of the mixture was adjusted to 3.0–4.0 (glass electrode) before extraction with three separate 50-ml. portions of recently distilled chloroform. The chloroform was withdrawn and evaporated until only a few ml. remained which were transferred to a small vial and taken up in agar.

Excellent pollen grain germination and pollen tube growth for the various species tested occurred on a medium composed of 1% agar and 10% sucrose. The pollen was distributed uniformly over 2 ml. of sterile medium in a sterile petri dish. A piece of moist filter paper was inserted inside the cover and the dish was placed in a darkroom at 25°C. All cultures were examined microscopically before being ground with glass, acidified and extracted. Occasionally the cultures older than 24 hrs. were found to be contaminated and were discarded.

Representative experiments for each type of pollen are presented in table 1. *Nicotiana* pollen has a relatively small amount of free hormone and no marked increase in amount occurs upon germination. The pollen of *Datura* contains twice as much free hormone as the pollen of *Nicotiana* but no increase in amount occurs following germination. Upon germination of the *Antirrhinum* pollen the growth hormone concentration increased to an amount which was three or four times that present in the

TABLE 1  
CONCENTRATIONS OF GROWTH HORMONES OBTAINED BY EXTRACTION OF POLLEN GRAINS  
AND POLLEN TUBES

SOURCE OF POLLEN	STAGE OF MATERIAL EXTRACTED	MO. OF POLLEN GRAINS	AVERAGE AVENA TEST CURVATURE	MICROGRAMS OF INDOLEACETIC ACID $\times 10^{-4}$ PER MO. OF POLLEN
<i>Nicotiana tabacum</i>	Grains	29.0	4.2 $\pm$ 1.2	0.7
	Grains	29.3	2.0 $\pm$ 0.8	0.3
	Tubes (15 Hrs.)	14.0	0.0	0.0
	Tubes (15 Hrs.)	30.0	0.0	0.0
	Tubes (25 Hrs.)	34.5	0.0	0.0
	Tubes (25 Hrs.)	35.0	8.6 $\pm$ 0.8	1.2
	Tubes (37 Hrs.)	29.2	2.0 $\pm$ 0.8	0.3
	Tubes (37 Hrs.)	28.7	5.0 $\pm$ 1.1	0.9
<i>Antirrhinum majus</i>	Grains	30.0	4.7 $\pm$ 0.9	1.1
	Tubes ( 8 Hrs.)	30.0	16.7 $\pm$ 1.2	3.9
	Tubes ( 8 Hrs.)	30.0	17.3 $\pm$ 1.8	4.0
	Tubes (14 Hrs.)	30.0	17.1 $\pm$ 1.8	4.0
	Tubes (14 Hrs.)	30.0	17.4 $\pm$ 1.4	4.0
	Tubes (14 Hrs.)	30.0	14.5 $\pm$ 0.7	3.4
	Tubes (25 Hrs.)	30.0	14.9 $\pm$ 1.5	3.5
	Tubes (25 Hrs.)	30.0	13.0 $\pm$ 1.1	3.0
<i>Cyclamen persicum</i>	Grains	11.0	3.9 $\pm$ 1.5	2.4
	Tubes ( 6 Hrs.)	12.0	3.4 $\pm$ 1.1	1.9
	Tubes ( 6 Hrs.)	13.0	7.9 $\pm$ 0.8	4.4
	Tubes (10 Hrs.)	12.0	4.7 $\pm$ 0.4	2.6
	Tubes (22 Hrs.)	12.0	7.2 $\pm$ 0.7	4.0
	Tubes (22 Hrs.)	12.0	9.9 $\pm$ 0.7	6.0
<i>Datura suaveolens</i>	Grains	53.0	11.3 $\pm$ 1.1	1.4
	Tubes ( 9 Hrs.)	70.0	16.5 $\pm$ 2.5	1.6
	Tubes ( 9 Hrs.)	63.0	14.3 $\pm$ 1.6	1.5
	Tubes (26 Hrs.)	62.0	16.4 $\pm$ 1.5	1.8

grain. The pollen grains of *Cyclamen* were the richest source of hormones. The concentration of free hormone in the germinated pollen was double or triple the concentration before germination. The considerable fluctuation in the concentration of free hormone was found to be characteristic for the pollen tube growth of both *Nicotiana* and *Cyclamen*.

*Growth Hormones in Pollen Grains Subjected to Hydrolysis.*—The increased concentrations of growth hormones in the germinated pollen of *Antirrhinum* and *Cyclamen* suggests the liberation of active hormones from inactive combinations during the growth of the pollen tube. The investigations of Skoog and Thimann,<sup>18</sup> Wildman and Gordon,<sup>19</sup> Gordon<sup>6</sup> and Avery, Berger and White<sup>1</sup> all indicate the existence of growth substances both as free, active entities and in bound, inactive combinations. To release the active hormones the pollen grains were subjected to acid and alkaline hydrolysis following the methods of Avery, Berger and White.<sup>1</sup> Thirty mg. of pollen grains were ground with powdered glass and trans-

ferred to a large test tube with 5 ml. 1.0 *N* NaOH or 10 ml. 0.1 *N* HCl. The mixtures were autoclaved 30 min. at 15-lb. pressure (120°C.) and allowed to cool. The pH was adjusted to 3.0–4.0 with 1.0 *N* HCl and the aqueous solutions were extracted with chloroform. The concentrations of free hormones following hydrolysis of the pollen materials are presented in table 2. A remarkable uniformity of yield following hydrolysis with

TABLE 2  
CONCENTRATIONS OF GROWTH HORMONES OBTAINED BY EXTRACTION OF POLLEN GRAINS  
FOLLOWING HYDROLYSIS

SOURCE OF POLLEN	EXP. NO.	TYPE OF HYDROLYSIS	AVERAGE AVENA TEST CURVATURE	MICROGRAMS OF INDOLEACETIC ACID $\times 10^{-4}$ PER MG. OF POLLEN
<i>Nicotiana tabacum</i>	1	None	0.0	0.0
		None	0.0	0.0
		None	0.0	0.0
		Alkali	30.5 $\pm$ 1.8	3.6
		Alkali	27.8 $\pm$ 1.5	3.3
		Alkali	30.4 $\pm$ 3.1	3.6
		Acid	0.0	0.0
		Acid	0.0	0.0
		Acid	0.0	0.0
<i>Antirrhinum majus</i>	1	None	4.0 $\pm$ 0.7	0.6
		None	0.0	0.0
		Alkali	31.6 $\pm$ 1.3	4.6
	2	Alkali	32.0 $\pm$ 0.9	4.7
		Alkali	19.7 $\pm$ 1.0	3.0
		Acid	20.2 $\pm$ 1.3	3.1
		Acid	19.2 $\pm$ 1.0	3.0
<i>Datura suaveolens</i>	1	None	22.4 $\pm$ 1.3	3.3
		Alkali	22.6 $\pm$ 1.4	3.3
		Alkali	24.4 $\pm$ 2.1	3.6
		Alkali	22.7 $\pm$ 1.5	3.3
	2	Alkali	28.4 $\pm$ 1.9	4.3
		Alkali	27.6 $\pm$ 1.8	4.2
		Acid	0.0	0.0
		Acid	0.0	0.0

alkali is demonstrated. These yields are the same as those obtained following germination of *Antirrhinum* and *Cyclamen* pollen but are much larger than the yields obtained from germinated pollen of *Nicotiana*. Although larger amounts of hormones were obtained in these experiments with pollen of *Datura* collected in June than were obtained in the germination tests with pollen collected in April, the hydrolysis of the material did not increase the yield of free hormone, which fact is in agreement with the demonstration that no marked change in concentration of growth substances occurred following germination of the pollen. Acid hydrolysis did not increase the yield of free hormone from *Nicotiana* pollen and decreased the yield from *Datura* pollen. Acid hydrolysis of the pollen of

*Antirrhinum* increased the yield of hormone as much as hydrolysis with alkali increased it. These data indicate that the hormones of the *Nicotiana* pollen are in a bound, inactive state and are only partially liberated during the germination of the grain. The hormones of *Antirrhinum* pollen are in a bound, inactive state for the most part but are liberated during germination. The hormones of *Datura* pollen are all present in the free, active form.

*Growth Hormones in Ovary Tissue.*—The hypothesis of the enzymatic activation of the auxin precursor in the ovary as a result of pollination made the assumption that a similar condition of active and inactive forms of the growth hormones occurred in the ovary as had been demonstrated by van Overbeek<sup>16</sup> for the coleoptile tip of maize seedlings. Extractions of dried ovary tissue of *Nicotiana* and *Antirrhinum* were made to investigate the occurrence of both free and bound forms of the hormones in the unpollinated pistil. The ovary tissue was dried *in vacuo* at 60°C. and ground to pass through an 80-mesh screen. Determinations were carried out with 20-mg. samples of this material.

The conversion of the bound form of the hormone to the free form by enzymatic digestion was investigated by dispersing the tissue in 10 ml. of  $\text{KH}_2\text{PO}_4$ -NaOH buffer solution of pH 8.0 with 3 mg. of a commercial pancreatic preparation (Fairchild). Numerous tests of this preparation have shown it to have no growth effects in the *Avena* test. Controls were prepared in which the enzyme preparation was omitted. The development of microorganisms in the digestions was prevented by adding 15 drops of toluol every 24 hrs. and tightly stoppering the flasks. Following incubation at 37°C. for 48 hrs., the pH of the mixtures was adjusted to 3.0–4.0 and the hormones were extracted from the mixtures with purified ether. The conversion of the bound form to the free hormone by hydrolysis with 1.0 *N* NaOH and 0.1 *N* HCl was determined as in the experiments with the pollen materials. The concentration of free hormone was determined by an ether extraction of the ovary tissue dispersed in acidified water for a period of 8 hrs. at 4°C.

The results of representative experiments with each type of tissue are presented in table 3. They show that the unpollinated pistil of *Nicotiana* contains no detectable free hormone but that a considerable quantity of bound hormone is present which can be converted to the active form merely by incubation in a medium of pH 8.0 and to a lesser degree by acid and alkali hydrolysis. The unpollinated pistil of *Antirrhinum* contains a small amount of free hormone but a much larger amount of bound hormone which can be converted to the active form by incubation in a medium of pH 8.0 and by acid hydrolysis but not appreciably by alkali hydrolysis.

*The Conversion of Bound Hormone to Free Hormone by Pollen Extracts.*—The demonstration of considerable quantities of bound hormone in the

TABLE 3

CONCENTRATIONS OF GROWTH HORMONES OBTAINED BY EXTRACTION OF OVARY TISSUE

SOURCE OF TISSUE	TREATMENT	AVERAGE AVENA TEST CURVATURE	MICROGRAMS OF INDOLEACETIC ACID $\times 10^{-4}$ PER MG. OF TISSUE (FRESH WT.)
<i>Nicotiana tabacum</i>	Tryptic digestion	37.3 $\pm$ 2.5	1.5
	Tryptic digestion	32.0 $\pm$ 1.9	1.3
	Control incubation	38.0 $\pm$ 1.5	1.5
	Acid hydrolysis	10.0 $\pm$ 0.2	0.4
	Acid hydrolysis	10.2 $\pm$ 0.7	0.4
	Alkali hydrolysis	12.4 $\pm$ 0.8	0.5
	Alkali hydrolysis	9.1 $\pm$ 1.0	0.4
	None	0.0	0.0
	None	0.0	0.0
<i>Antirrhinum majus</i>	Tryptic digestion	26.2 $\pm$ 2.0	1.3
	Control incubation	19.5 $\pm$ 1.9	1.0
	Control incubation	14.3 $\pm$ 1.0	0.7
	Acid hydrolysis	14.0 $\pm$ 0.8	0.7
	Alkali hydrolysis	2.3 $\pm$ 0.4	0.1
	Alkali hydrolysis	3.9 $\pm$ 0.7	0.2
	None	5.4 $\pm$ 0.5	0.3
	None	3.1 $\pm$ 0.8	0.2

TABLE 4

THE CONVERSION OF BOUND HORMONE TO FREE HORMONE IN OVARY TISSUE OF NICOTIANA BY POLLEN EXTRACTS

EXP. NO.	MEDIUM	PREPARATION	AVERAGE AVENA TEST CURVATURE
1	Distilled water, pH 8.3	Ovary tissue + pollen extract	22.8 $\pm$ 1.2
		Ovary tissue	0.0
		Ovary tissue	0.0
		Pollen extract	0.0
		Pollen extract	0.0
	Buffer solution, pH 5.9	Ovary tissue + pollen extract	27.0 $\pm$ 2.6
		Ovary tissue + pollen extract	20.0 $\pm$ 1.1
		Ovary tissue	0.0
		Ovary tissue	0.0
		Pollen extract	0.0
2	Buffer solution, pH 5.9	Pollen extract	0.0
		Ovary tissue + pollen extract	15.0 $\pm$ 1.3
		Ovary tissue + pollen extract	14.7 $\pm$ 1.4
		Ovary tissue	5.5 $\pm$ 1.2
		Ovary tissue	4.5 $\pm$ 1.1
	Buffer solution, pH 8.0	Pollen extract	0.0
		Pollen extract	0.0
		Ovary tissue + pollen extract	22.7 $\pm$ 1.6
		Ovary tissue + pollen extract	40.2 $\pm$ 3.2
		Ovary tissue	34.1 $\pm$ 1.7
		Ovary tissue	34.8 $\pm$ 1.7
		Pollen extract	0.0



ovary of *Nicotiana* and *Antirrhinum* suggested the investigation of substances in pollen which might bring about the activation of the hormones as hypothesized by van Overbeek, Conklin and Blakeslee. Extracts were made by grinding 0.1 gm. of *Nicotiana* pollen grains with glass dispersing the material in distilled water and toluol, and agitating the mixture mechanically for 37 hrs. at 13°C. The suspension was centrifuged to remove the glass and pollen debris and 16 ml. of a yellowish, slightly turbid extract were obtained. Twenty mg. of dried ovary tissue of *Nicotiana* (unpollinated pistils) were dispersed in 10-ml. portions of distilled water and  $\text{KH}_2\text{PO}_4$ -NaOH buffer solutions of pH 8.0 and 5.9. Two ml. of the pollen extract were added to one set of dispersions, 2 ml. of distilled water were added to a control set and 2 ml. of the pollen extract were added to 10 ml. of water and buffer solutions which did not contain ovary tissue. All mixtures were incubated at 37°C. for 24 hrs. after 20 drops of toluol had been added and the flasks tightly stoppered. After incubation the pH of the mixtures was adjusted to 3.0–4.0 and extractions were made with ether.

The results of two experiments presented in table 4 show that in distilled water and buffer solution of pH 5.9 the mixture of ovary tissue and pollen extract yielded large quantities of free hormones whereas the ovary tissue alone and the pollen extract alone yielded none or little free hormone. In buffer solution of pH 8.0 the same yields of free hormones were obtained from the ovary tissue alone as were obtained from the mixture of ovary tissue and pollen extract. The conversion of the bound form of the hormone to the free form in an alkaline medium has been observed previously (see table 3). These experiments demonstrate that the extract of the pollen contains a substance or substances which can convert the bound hormones in the dried ovary tissue to free hormones.

*Discussion and Interpretation of Experimental Results.*—Since the pollen grains, pollen tubes and ovaries have all been shown to contain growth substances, the source of the diffusible hormones detected in the ovary following pollination and fertilization can be established only by a comparison of the amounts in the pollen and ovary tissue. Buchholz<sup>4</sup> has stated that 600–900 pollen grains may be regarded as a normal abundant pollination in *Datura* and van Overbeek, Conklin and Blakeslee cite this figure to support their view that the pollen has insufficient hormones to cause fruit development. Thus 1000 pollen grains would be a liberal figure for pollination. Determinations of hormone concentrations were made on a weight basis and the calculation of amounts of hormones involved in a normal pollination requires estimates of the number of grains per unit weight of pollen. Heyl<sup>5</sup> while studying the chemical composition of pollen estimated that there are 610 millions of grains in one gram of ragweed pollen and Ulrich<sup>14</sup> estimated 173 millions of grains per gram of



ragweed pollen. In this study estimates of the number of grains per gram of pollen were made by dispersing a weighed amount of pollen in a definite volume of 50% glycerol and water after wetting the grains with a drop of chloroform. Duplicate aliquots of the suspension were placed on a glass slide under a cover glass and counted with the aid of a mechanical stage. The pollen grain of *Nicotiana* has an average volume of 14,500 cubic microns and there are approximately 60 millions of grains per gram of fresh pollen. The pollen grain of *Antirrhinum* has an average volume of 5300 cubic microns and there are approximately 170 millions of grains per gram of fresh pollen.

Small yields of growth hormones were obtained from *Nicotiana* pollen grains and tubes but approximately  $4.0 \times 10^{-4}$  microgram of indoleacetic acid per milligram of pollen was obtained following alkali hydrolysis and as this concentration corresponds well with maximum yields from other types of pollen it will be acceptable as the maximum concentration in *Nicotiana* pollen. Calculation reveals that the maximum concentration of growth hormones in the pollen of a normal pollination is then  $6 \times 10^{-4}$  microgram of indoleacetic acid. In experiments with *Nicotiana tabacum* reported elsewhere (Muir<sup>11</sup>) it was found that immediately following fertilization there appear sufficient diffusible growth hormones in the ovary to produce curvatures of 30 degrees in the *Avena* test, a concentration of approximately  $6 \times 10^{-4}$  microgram of indoleacetic acid, 100 times as much hormone as is contained in the pollen of a normal pollination. Similarly, following pollination there appear sufficient diffusible growth hormones in apical and basal portions of the style to produce curvatures of 10 degrees, a concentration of approximately  $2 \times 10^{-4}$  microgram of indoleacetic acid, 30 times as much growth hormones as are contained in the pollen. Maximum yields of hormones from the ovary tissue of unpollinated *Nicotiana* pistils were  $1.5 \times 10^{-4}$  microgram of indoleacetic acid per milligram of fresh tissue. The ovary with an average fresh weight of 34 mg. thus contained approximately  $5 \times 10^{-3}$  microgram of indoleacetic acid, 800 times as much hormone as was found in the pollen. Yields of hormones obtained by extraction of unpollinated pistils were 8 times as great as the yields obtained by diffusion of fertilized ovaries.

Calculation of the amount of growth hormones in the pollen of a normal pollination for *Antirrhinum* gives a value of approximately  $2 \times 10^{-4}$  microgram of indoleacetic acid. The maximum yield of hormones from the ovary tissue of unpollinated *Antirrhinum* pistils was  $1.3 \times 10^{-4}$  microgram of indoleacetic acid per milligram of tissue. The ovary with an average fresh weight of 8 mg. thus contained approximately  $1 \times 10^{-3}$  microgram of indoleacetic acid, 500 times as much hormone as contained in the pollen.

The above comparisons of the amounts of growth hormones in the

pollen and ovary tissue and the demonstration that incubation of water extracts of pollen with ovary tissue yields large amounts of free growth hormones establish the fact that changes in concentration of active hormones in the pistil associated with pollination and fertilization are the result of a substance or substances, other than growth hormones, brought into the pistil by the pollen tubes.

The identity of the effective substance in pollen will be indicated more definitely when the mechanism of free auxin formation in the ovary following fertilization has been established. The investigations of Skoog and Thimann<sup>18</sup> and Wildman and Gordon<sup>19</sup> suggest that in some tissues the inactive growth hormones are bound to proteins and thus the effective substance in pollen may be part of an enzyme system which brings about the release of active hormone from protein combinations. Recently Bonner and Wildman<sup>3</sup> have suggested that the hormone protein complex in the leaves of spinach is an active entity with the enzymatic properties of a phosphatase and that the hormone, indoleacetic acid, is formed from tryptophane. The significance of phosphatase in the metabolism of reproduction in plants is indicated by the data of Ignatieff<sup>9</sup> who found that the phosphatase activity and the total phosphorous content of pistils and stamens are greater than in any other part of the flowering plant. In plant tissues other than leaves the inactive growth hormones may be true storage forms as indicated by the investigations of Gordon<sup>6</sup> on the hormone and protein combinations in wheat grains and the investigations of Berger and Avery<sup>2</sup> on the inactive hormone in maize endosperm. The possibility thus exists that the pollen substance is a coenzyme or activator of enzymatic systems present in the ovary which liberate active hormones from the storage forms.

*Summary.*—1. Determinations of growth hormone concentrations in pollen grains and pollen tubes have shown that free hormones are present in the pollen grains and that variable amounts are present during pollen tube growth. Larger quantities are present in a bound form that can be activated by alkali hydrolysis in the pollen of *Nicotiana* and by both acid and alkali hydrolysis in the pollen of *Antirrhinum*. The growth hormones in the pollen of *Datura* are all present in the active form. Maximum yields obtained by various procedures are in agreement.

2. Little or no growth hormone is present in the active form in dried ovary tissue of *Nicotiana* and *Antirrhinum* but large quantities are present in an inactive form that can be activated most completely by incubation at pH 8.0 and less completely by acid or alkali hydrolysis. A water extract of *Nicotiana* pollen contains a substance or substances which can bring about the conversion of the inactive growth hormones in the dried ovary tissue of *Nicotiana* to the active forms in an acid medium *in vitro*.

3. Comparisons of the amounts of growth hormones contained in the

pollen of a normal pollination with the amounts of diffusible hormones which appear in the ovary immediately after pollination and fertilization and with the amounts of inactive hormones in the ovary indicate that the changes in growth hormone concentrations following fertilization are the result of a substance in the pollen other than the growth hormones. It is suggested that this substance is part of an enzyme system.

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## NUTRITIONAL LIFE HISTORY AS INFLUENCED BY DIETARY ENRICHMENTS. II. BODY WEIGHT AND BODY CALCIUM IN CASES OF PROTEIN-ACCELERATED GROWTH\*

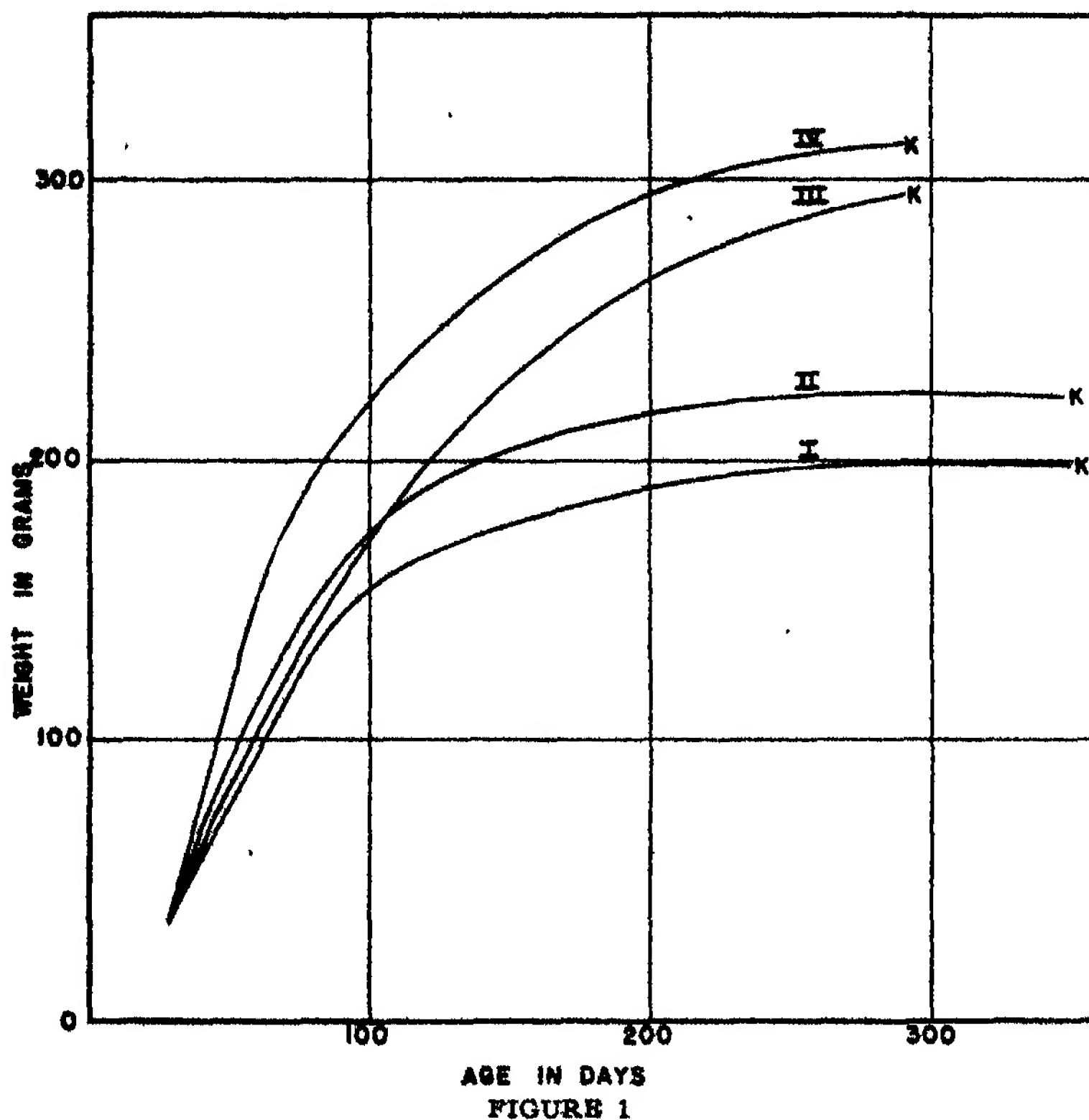
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Communicated August 20, 1947

In a previous paper<sup>1</sup> we have reported that when a diet of about minimal adequacy in protein and calcium content was enriched in protein by the addition of poultry meat, growth was accelerated but calcium retention did not keep pace. During the resulting period of rapid growth with relatively low calcium content of body a minority of the experimental animals showed symptoms suggestive of calcium deficiency, while the

majority showed no visible injury and continued to make increased gains in body weight up to the age of 84 days as shown by the tabulated data of our preceding paper.



Smoothed curves representing body weights: I, average of three females receiving basal diet *ad lib*; II, average of three females receiving the same plus 60 g. per week of poultry meat; III, of a male receiving basal diet *ad lib*; IV, of a litter-mate male receiving the same plus 60 g. per week of poultry meat. The date of killing for analysis is indicated by K.

As the protein-accelerated rats reached about the average adult size for the animals of the Columbia colony, their weight curves flattened off while the smaller animals which had received the basal diet only, continued to grow. But at about one year of age when the slower-growing rats were also reaching their final adult size, the animals which had the protein-enriched diet still averaged larger than the controls which had received the basal diet only. This difference, while not large nor invariable, is probably significant. The curves shown in figure 1 are representative of our general experience on this point. These particular animals were then

killed and analyzed for body calcium at about one year of age, with the results shown in table 1.

TABLE 1  
COMPARISON OF CALCIUM CONTENTS OF ADULT RATS FED BASAL DIET 16 ALONE OR WITH THE ADDITION OF 60 GM. PER WEEK OF POULTRY MEAT (DIET 16 P 10)

	FROM DIET 16	FROM DIET 16 P 10
Males (litter mates)		
Age, days	290	290
Weight, g.	295	314
Body calcium, g.	1.87	1.76
Body calcium, %	0.67	0.60
Females (average of 3 in each case)		
Age, days	354	348
Weight, g.	199	221
Body calcium, g.	1.99	1.89
Body calcium, %	1.08	0.93

It will be seen that at full adulthood the animals which had received the protein-enriched diet were still ahead in body weight and behind (their controls) in body calcium. This is the more noteworthy in that the animals which had received the basal diet only contained both larger amounts and higher percentages of calcium in their bodies.

The larger number of the animals which were given this dietary enrichment are being continued, as are their controls, in the hope of carrying the comparison throughout their complete life histories.

\* The work recorded in this paper and in paper I of this series was aided by grants from the John and Mary R. Markle Foundation.

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## DEPLETION MUTATION IN *SACCHAROMYCES*\*

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An  $\alpha$  mating type galactose-, maltose-fermenting haplophase segregant of *Saccharomyces cerevisiae* was subjected to mustard gas treatment by Tatum and Reaume (Tatum and Reaume, in ms.) and produced an adenine-dependent mutant with pink colonies. The symbol ad(P) indicates the adenine-dependent variant producing pink colonies; ad indicates the same allele carried by a white phenotype. The symbol AD indicates the dominant allele; no secondary symbol is necessary for



these are always white. The adenine-dependent pink (ad(P)) culture was shown to be a gene mutation by hybridizing it with an adenine-independent white (AD) haplophase of *S. cerevisiae*.

	MATING		HYBRID	SEGREGANTS			
	Haploid	× haploid	Diploid	Haploid	Haploid	Haploid	Haploid
Gene	ad(P)	× AD	ad(P)/AD	ad(P)	ad(P)	AD	AD
Color	Pink	× White	White	Pink	Pink	White	White

Forty-six asci were dissected from the white hybrid and in 42 asci, two white and two pink cultures arose from each ascus, proving that gene mutation was involved (table 1). In these 42 asci the pink cultures were adenine-dependent and the white cultures were adenine-independent.

TABLE 1  
GENETICAL ANALYSIS OF ASCI FROM VARIOUS CROSSES INVOLVING PINK AND WHITE COLOR, AND ADENINE AND METHIONINE SYNTHESIS

MATINGS		NO. ASCI	SEGREGANTS				COLOR
			A	B	C	D	
ad(P)MET × AD MET pink            white		42	ad(P)	ad(P)	AD	AD	2 pink:2 white
		1	ad(P)	ad	AD	AD	1 pink:3 white
		2	ad	ad	AD	AD	0 pink:4 white
		1	ad(P)	ad(P)	ad(P)	AD	3 pink:1 white
ad(P)MET × AD met pink            white		7	ad(P)MET	ad(P)MET	AD met	AD met	2 pink:2 white
		5	ad met	ad met	AD MET	AD MET	0 pink:4 white
		23	ad(P)MET	ad met	AD met	AD MET	1 pink:3 white
ad met × AD MET white    white		7	ad(P)MET	ad(P)MET	AD met	AD met	2 pink:2 white
		5	ad met	ad met	AD MET	AD MET	0 pink:4 white
		27	ad(P)MET	ad met	AD met	AD MET	1 pink:3 white
ad(P)MET × ad(P)MET pink            pink		6	ad(P)MET	ad(P)MET	ad(P)MET	ad(P)MET	4 pink:0 white
ad met × ad met white    white		3	ad met	ad met	ad met	ad met	0 pink:4 white
ad(P)MET × AD MET pink            white		7	ad(P)MET	ad(P)MET	AD MET	AD MET	2 pink:2 white
ad MET × ad(P)MET white    pink		11	ad(P)MET	ad(P)MET	ad(P)MET	ad(P)MET	4 pink:0 white
ad MET × ad(P)MET white    pink		10	ad(P)MET	ad(P)MET	ad(P)MET	ad(P)MET	4 pink:0 white
ad MET × ad met white    white		8	ad(P)MET	ad(P)MET	ad met	ad met	2 pink:2 white
ad MET × AD met white    white		1	ad(P)MET	ad(P)MET	AD met	AD met	2 pink:2 white
		5	ad(P)MET	ad met	AD met	AD MET	1 pink:3 white

Three asci produced fewer than the expected number of pink segregants. One contained three white and one pink culture; the pink segregant and one white culture were adenine-dependent. Two asci produced 4 white cultures; two were adenine-dependent and two were adenine-independent. These exceptional adenine-dependent white cultures will be discussed in detail below.

From one ascus more than the expected number of pink cultures was



obtained (three pink and one white culture) and the three pink segregants were adenine-dependent. Most of the adenine-dependent cultures adapt after four days and grow in the adenine-deficient medium. The differences in growth in adenine-deficient and adenine-containing medium are generally diagnostic on the second and third days; but growth of the so-called adenine-dependent cultures in the adenine-deficient medium becomes fairly dense on the sixth and seventh day. The extra pink culture may have been slow in adapting to adenine synthesis due to some other deficiency.

The hybrid was also heterozygous for mating type, galactose-, maltose- and melibiose-fermentation and there were no exceptions of these characters to Mendelian segregation.

The hybrid from which the 46 asci were analyzed was homozygous for genes controlling the synthesis of methionine, so that relatively adequate amounts of methionine were available to all four segregants. Tatum and Reaume also discovered a methionine-dependent mutant produced by mustard gas treatment and this gene was introduced into the stock by a series of matings. A hybrid heterozygous for adenine-dependence and methionine-dependence was produced. Analysis of 74 asci from the reciprocal crosses  $ad(P) MET \times AD met$  (pink  $\times$  white) and  $ad met \times AD MET$  (white  $\times$  white) revealed that the development of pink color required methionine. The adenine-dependent *white* cultures were also methionine-dependent and all the adenine-dependent *pink* cultures were methionine-independent proving that methionine was required for the development of the pink color.

I have pointed out that a doubly heterozygous hybrid produces only three kinds of asci and that the frequency of these three types can be used to detect linkage of either of the genes with each other or with their respective centromeres. The data in table 1, show that the two reciprocal matings, one producing 35 and the other producing 39 asci, both follow the same pattern and the three kinds of asci are present with a total frequency of 14:10:50, which is statistically equivalent to a 1:1:4 ratio, proving that the two genes are not linked. Asci containing 2 pink:2 white; 0 pink:4 white; 1 pink:3 white, correspond to the same three categories and reveal that pink is produced as a result of a two-factor interaction.

Six asci from a pink by pink hybrid produced four pink cultures from each ascus. Three asci dissected from a homozygous adenine-dependent, methionine-dependent hybrid produced only white cultures. Seven asci from an  $ad(P)MET \times AD MET$  back-cross hybrid produced 2 pink and 2 white per ascus. This further confirms the regular segregation of the two genes.

Adenine-dependence is the effect of the action of a single gene; pink pigment is a correlated effect which depends on the synergistic effect of

other genes as well. Pink pigment is apparently produced following the interaction of a precursor of adenine and an excess of methionine plus other substances. Pigment is usually produced in organisms incapable of completing the synthesis of adenine and capable of producing a considerable amount of methionine. The variation in intensity of color in different pink organisms indicates that many other factors affect color intensity.

*False Mutations.*—Some of the adenine-dependent, methionine-dependent *white* cultures were transferred to peptone agar to which an excess of methionine had been added. Pink cultures appeared thus confirming the dependence of the pink character on the presence of methionine. Added methionine did not induce the development of a pink color in any of the adenine-independent *white* organisms. When the cultures arising from homozygous *ad met* stocks were grown on agar, numerous small secondary pink papillae often appear suggesting local accumulation of sufficient methionine to produce the pink color.

Variations in bacteria following environmental changes have often been called "mutations" but the present experiments show that variations may also be due to a deficiency either of external or internal origin which prevents the development of the characteristic phenotype on a deficient medium. False "mutations" from pink to white may appear when growth occurs in the absence of sufficient methionine to insure the production of the pink color; many pink cultures have white borders which may arise when the supply of methionine in the medium becomes exhausted. Transfer of these false "mutants" to a medium containing sufficient methionine may result in a false "reverse mutation" from white to pink without any change occurring in the gene itself.

*Depletion Mutation.*—Tatum and Reaume discovered that white (*ad MET*) variant colonies which retain their methionine synthesizing ability often arise from pink cultures on vegetative propagation—a fact which we have confirmed, and which they will discuss in greater detail elsewhere. Numerous white variants of the original pink have appeared. These white variants are *ad MET* like the white variants of Tatum and Reaume. Similar white segregants arising in the pink pedigree were subjected to genetical analysis.

Two hybrids of one of the exceptional adenine-deficient methionine-sufficient (*ad MET*) *white* cultures (obtained among the 46 asci from the first hybrid in table 1) by standard pinks, *ad(P)MET*, produced 21 asci containing 4 pink cultures each. Eight asci from a hybrid of the exceptional white (*ad MET*) by a standard adenine-dependent, methionine-dependent white produced two pink and two white cultures per ascus; the white cultures were methionine-dependent. Six asci of an exceptional white (*ad MET*) by an adenine-independent, methionine-dependent white

produced the progeny that would be expected if the exceptional white were a normal pink. This analysis indicates that the inability of these exceptional adenine-dependent, methionine-independent cultures to produce the pink pigment was due to some mechanism which is restored to activity following hybridization.

The following hypothesis is invoked to explain the effect of outcrossing in restoring the pink color. Pink depends upon the presence of the two genes *ad* and *MET* plus some other substances (*X*, *Y*, *Z*, etc.). The substance *X* is an essential component of gene *X* which has no other components besides *X*. Continuous production of pink exhausts the supply of *X* and results in the "running out" of the character. The stock to which the outcross is made carries gene *X* with an intact supply of the *X* component for since the stock does not produce pink it does exhaust its supply of the *X* substance. The outcross automatically restores the *X* substance and reestablishes the pink color. Other stocks may become white because *Y* or *Z* substances are exhausted. Mutations from pink to white are not the result of a drastic change in genotype but merely the result of the exhaustion of some gene component easily supplied by outcrossing to any normal stock.

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## WIND-DRIVEN CURRENTS IN A BAROCLINIC OCEAN; WITH APPLICATION TO THE EQUATORIAL CURRENTS OF THE EASTERN PACIFIC\*

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1. *Introduction.*—Permanent ocean currents are computed from the observed distribution of density on the assumptions (1) that the horizontal pressure gradient is balanced by the Coriolis force (the deflecting force of the earth's rotation) and (2) that the horizontal velocities and the hori-

zontal pressure gradient vanish at a moderate depth below the sea surface. The second condition can be fulfilled only in a baroclinic system, that is, in a system in which the isosteric surfaces intersect the isobaric surfaces.

In the computation of currents acceleration and frictional forces are neglected. Experience indicates that the computations lead to nearly correct results, implying that accelerations and frictional forces are small, but since friction is not entirely lacking, energy must be supplied to the ocean in order to maintain the permanent currents and the corresponding permanent distribution of mass. This energy can be supplied by the effects of heating and cooling or by the stress which the prevailing winds exert on the sea surface. Of the sources the latter is generally considered to be the more important. We shall examine effects of the wind stress only, taking into account that the ocean waters in motion represent a baroclinic system.

Ekman<sup>1</sup> and Stockmann<sup>2</sup> have examined the currents which develop in a *homogeneous* ocean under the influence of a stress exerted on the free surface, and Fjeldstad<sup>3</sup> has solved a special problem dealing with baroclinic conditions. If the general problem for a baroclinic ocean could be solved, knowledge of the wind stress alone would enable us to compute the permanent ocean currents, provided the effects of heating and cooling were negligible. A treatment of this general problem would present great mathematical difficulties because it would require the introduction of lateral frictional stresses and complete boundary conditions. Here we shall deal with the special case of equatorial currents in a region where lateral stresses can be neglected, boundary conditions are relatively simple, wind systems are semipermanent, and where our results imply that effects of heating and cooling, if present, need not be considered explicitly.

The striking feature of the currents of the equatorial regions is that imbedded between the currents which flow toward the *west* under the influence of the prevailing trade winds equatorial counter currents flow toward the *east*. In the Pacific and Atlantic Oceans the counter current is particularly well developed in the eastern parts of the oceans where it is located north of the equator, its axis coinciding approximately with the location of the equatorial calm belt which is found further to the north in summer than in winter. In the Indian Ocean the counter current is found to the south of the equator, but in the northern winter only.

Our specific problem is to determine whether the equatorial currents, including the counter currents, can be accounted for on the basis of our knowledge of the wind stress only. This problem was first approached by Montgomery and Palmén,<sup>4</sup> but Stockmann<sup>2</sup> has shown that they did not treat it in a sufficiently general manner. Stockmann's theoretical results, however, are not applicable to the conditions in the ocean because he assumed homogeneous water, but a similar analysis for a baroclinic

system leads to a remarkable agreement between theoretical conclusions and observed conditions.

2. *Theory.*—The ocean waters are so nearly in hydrostatic equilibrium that at any depth the pressure,  $p$ , can be determined by a numerical integration of the hydrostatic equation:

$$dp = g\rho dz \quad (1)$$

provided that the density,  $\rho$ , is known from observations. In equation (1) and in the following equations the  $z$ -axis is positive downwards.

Neglecting lateral stresses the equations of horizontal motion can be written:

$$\begin{aligned} \frac{\partial u}{\partial t} + u \frac{\partial u}{\partial x} + v \frac{\partial u}{\partial y} &= -\frac{1}{\rho} \frac{\partial p}{\partial x} + \lambda v + \frac{1}{\rho} \frac{\partial}{\partial z} \left( A \frac{\partial u}{\partial z} \right) \\ \frac{\partial v}{\partial t} + u \frac{\partial v}{\partial x} + v \frac{\partial v}{\partial y} &= -\frac{1}{\rho} \frac{\partial p}{\partial y} - \lambda u + \frac{1}{\rho} \frac{\partial}{\partial z} \left( A \frac{\partial v}{\partial z} \right) \end{aligned} \quad (2)$$

where  $u$  and  $v$  are the horizontal velocity components in a rectilinear coordinate system,  $\lambda = 2\omega \sin \varphi$  ( $\omega$  the earth's angular velocity of rotation,  $\varphi$  the latitude, taken positive to the north of the equator), and  $A$  is the eddy viscosity.

We shall assume stationary conditions,

$$\frac{\partial u}{\partial t} = \frac{\partial v}{\partial t} = 0, \quad (3)$$

and shall neglect the non-linear terms, the field accelerations:

$$u \frac{\partial u}{\partial x} + v \frac{\partial u}{\partial y} = u \frac{\partial v}{\partial x} + v \frac{\partial v}{\partial y} = 0, \quad (4)$$

thereby placing severe restrictions upon the possible lateral boundary conditions. Equations (2) reduce to:

$$\begin{aligned} \frac{\partial p}{\partial x} &= \lambda \rho v + \frac{\partial}{\partial z} \left( A \frac{\partial u}{\partial z} \right) \\ \frac{\partial p}{\partial y} &= -\lambda \rho u + \frac{\partial}{\partial z} \left( A \frac{\partial v}{\partial z} \right) \end{aligned} \quad (5)$$

stating that the horizontal pressure gradient is balanced by the Coriolis force and frictional stresses exerted on horizontal surfaces. In *homogeneous* water the horizontal pressure gradient is independent of depth but in a *baroclinic* system varies with depth. In the ocean it generally vanishes at



a moderate depth, less than that to the bottom. We define a function  $P$  by the integrals

$$\frac{\partial P}{\partial x} = \int_0^d \frac{\partial \rho}{\partial x} dz, \quad \frac{\partial P}{\partial y} = \int_0^d \frac{\partial \rho}{\partial y} dz \quad (6)$$

where  $d$  is equal to or greater than the depth at which the horizontal pressure gradient becomes zero. The function  $P$ , which is closely related to the  $P$ -function introduced by Ekman,<sup>5</sup> can be computed from the observed vertical distribution of density at a single oceanographic station, using equation (1).

The horizontal velocity must vanish at or above the depth  $d$ . The integrals

$$M_x = \int_0^d \rho u dz, \quad M_y = \int_0^d \rho v dz \quad (7)$$

represent therefore the components of the *net* mass transport by the currents.

Integrating equations (5) from 0 to  $d$ , and introducing the horizontal boundary conditions:

$$\begin{aligned} \left( A \frac{\partial u}{\partial z} \right)_0 &= -\tau_x, & \left( A \frac{\partial u}{\partial z} \right)_d &= 0 \\ \left( A \frac{\partial v}{\partial z} \right)_0 &= -\tau_y, & \left( A \frac{\partial v}{\partial z} \right)_d &= 0 \end{aligned} \quad (8)$$

where  $\tau_x$  and  $\tau_y$  are the components of the wind stress, we obtain:

$$\frac{\partial P}{\partial x} = \lambda M_y + \tau_x \quad (9a)$$

$$\frac{\partial P}{\partial y} = -\lambda M_x + \tau_y \quad (9b)$$

The terms in equations (9) are well known in oceanography. Omitting the stress components the equations give the mass transport related to the distribution of density, or assuming homogeneous water in hydrostatic equilibrium ( $\partial P/\partial x = \partial P/\partial y = 0$ ) they give the mass transport by pure wind currents. Equations (9) have been used by Defant<sup>6</sup> for computing the wind stress from oceanographic observations, including direct measurements of currents, but they have not been applied to other problems.

For application to other problems we add the equation:

$$\frac{\partial M_x}{\partial x} + \frac{\partial M_y}{\partial y} = 0 \quad (10)$$

which is obtained by integration of the equation of continuity, assuming



that the vertical velocity is zero at the free surface and at the depth  $d$ . The three equations (9a), (9b) and (10) can be considered as relating the three unknown quantities,  $P$ ,  $M_x$ , and  $M_y$ , to the known wind stress. Consequently, the distribution of density, as described by the partial derivatives of  $P$ , and the mass transport by the corresponding currents can be expressed as functions of the stress.

In applying equations (9) and (10) to equatorial currents we place the positive  $x$ -axis toward the east and the positive  $y$ -axis toward the north, and let  $y = 0$  at the equator ( $\varphi = 0$ ). Since

$$dy = R d\varphi \quad (11)$$

where  $R$  is the radius of the earth:

$$\frac{\partial \lambda}{\partial x} = 0, \quad \frac{\partial \lambda}{\partial y} = \frac{2\omega \cos \varphi}{R}, \quad \frac{\partial^2 \lambda}{\partial y^2} = -\frac{2\omega \sin \varphi}{R^2} \quad (12)$$

Differentiating equation (9a) with respect to  $y$  and (9b) with respect to  $x$ , subtracting and taking equations (10) and (12) into account, we obtain

$$M_y \frac{\partial \lambda}{\partial y} + \left( \frac{\partial \tau_x}{\partial y} - \frac{\partial \tau_y}{\partial x} \right) = 0 \quad (13)$$

In the trade-wind belt of the eastern Pacific the term  $\partial \tau_y / \partial x$  is so small that with good approximation:

$$M_y = \frac{\partial \tau_x}{\partial y} / \frac{\partial \lambda}{\partial y} = -\frac{\partial \tau_x}{\partial y} \frac{R}{2\omega \cos \varphi} \quad (14)$$

Introducing equation (14) in (9a):

$$\frac{\partial P}{\partial x} = -\frac{\partial \tau_x}{\partial y} R \tan \varphi + \tau_x \quad (15)$$

or, writing differences on the left-hand side:

$$\frac{\Delta P}{\Delta x} = -\frac{\overline{\partial \tau_x}}{\partial y} R \tan \varphi + \bar{\tau}_x \quad (16)$$

where averages over the distance  $\Delta x$  are indicated by bars.

From equations (10) and (14) follows

$$\frac{\partial M_x}{\partial x} = \frac{1}{2\omega \cos \varphi} \left( \frac{\partial \tau_x}{\partial y} \tan \varphi + \frac{\partial^2 \tau_x}{\partial y^2} R \right) \quad (17)$$

When integrating equation (17) from 0 to  $\Delta x$  we shall assume a north-south vertical boundary at  $x = 0$  at which the kinematic boundary condition  $u_0 = 0$  must be satisfied in the form  $M_x = 0$ . We obtain:

$$M_z = \frac{\Delta x}{2\omega \cos \varphi} \left( \frac{\partial \tau_z}{\partial y} \tan \varphi + \frac{\partial^2 \tau_z}{\partial y^2} R \right) \quad (18)$$

Equation (18) cannot hold at a second north-south boundary at, say,  $x = L$ , at which the condition  $M_L = 0$  must be satisfied. This inadequacy of our solution is due to the neglect of the field accelerations (eq. 4). Attempts will be made to find more general solutions and to study other special cases.

Substituting equation (18) in (9b):

$$\frac{\partial P}{\partial y} = -\Delta x \tan \varphi \cdot \left( \frac{\partial \tau_z}{\partial y} \tan \varphi + \frac{\partial^2 \tau_z}{\partial y^2} R \right) + \tau_y \quad (19)$$

Equations (15) or (16) and (19), together with (14) and (18), represent in our special case the relationships of the distribution of mass and the corresponding mass transport to the wind stress. The validity of our results can be tested where suitable observations are available.

3. *Discussion.*—The available oceanographic observations comprise (1) a line of 8 stations between latitudes  $22^\circ\text{N}$  and  $10^\circ\text{S}$ , longitudes  $137^\circ\text{W}$  and  $162^\circ\text{W}$ , occupied by the *Carnegie* between October 21 and November 4, 1929 (Fleming<sup>7</sup>); (2) a line of 12 stations between latitudes  $6^\circ\text{N}$  and  $9^\circ\text{S}$ , longitudes  $80^\circ\text{W}$  and  $108^\circ\text{W}$ , occupied by the *Carnegie* between October 26 and November 21, 1928; and (3) a line of 8 stations between latitudes  $9^\circ\text{N}$  and  $21^\circ\text{N}$ , longitudes  $87^\circ\text{W}$  and  $109^\circ\text{W}$ , occupied by the *Bushnell* between March 18 and March 24, 1939 (Sverdrup<sup>8</sup>). From the observations at each of these stations the value of the function  $P$  was computed by integrating to a depth of 1000 meters. From all data the ratio  $\Delta P/\Delta x$  was found, and from the *Carnegie* section in mid-ocean  $\partial P/\partial y$  was derived.

Wind observations comprise (1) monthly wind roses for 5-degree squares published in the Pilot Charts of the North and South Pacific, giving the percentage of winds from different directions and the corresponding average wind force (on the Beaufort scale) and (2) compilations of frequencies of winds of different forces in the "Atlas of Climatological Charts of the Oceans."<sup>9</sup> From the wind data the average wind stresses in October and November were computed, using the relationship

$$\tau = \gamma^2 \rho' U^2$$

where  $\gamma^2$  is the resistance coefficient,  $\rho'$  the density of the air, and  $U$  the wind speed as estimated at a height of about 10 meters. At wind force 3 Beaufort or less the sea surface was assumed to be hydrodynamically smooth, with a resistance coefficient of about  $0.8 \times 10^{-3}$ , decreasing somewhat with increasing wind speed. At wind force 4 Beaufort and higher a constant value,  $\gamma^2 = 2.6 \times 10^{-3}$ , was used, corresponding to a hydrodynamically rough surface (Rossby<sup>10</sup>). The manner in which all computa-

tions were carried out will be described elsewhere by the author and R. O. Reid, who has prepared the figures in this paper.

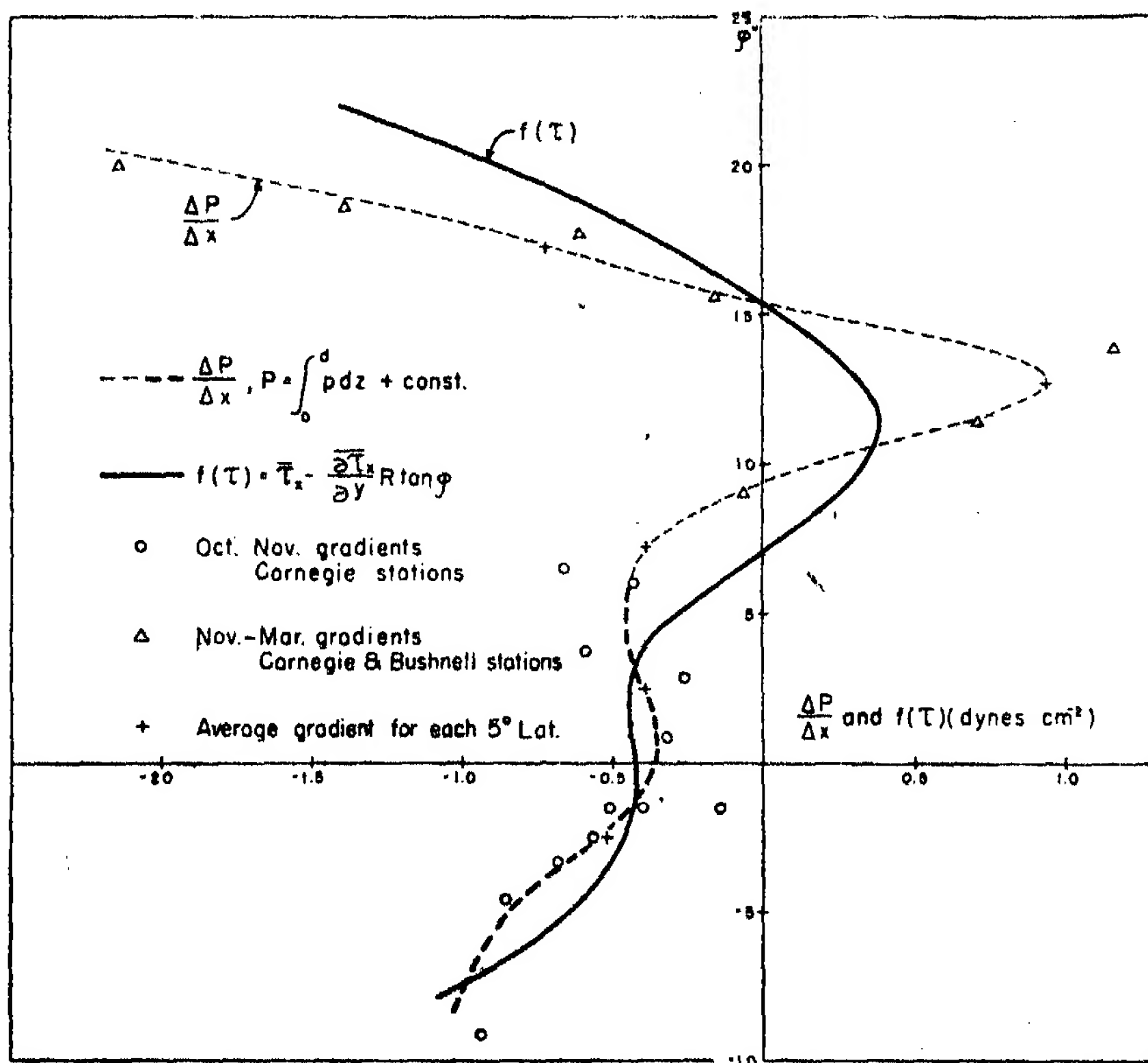


FIGURE 1

In figure 1 the terms of equation (16) are shown as functions of latitude. The curve that represents the left-hand term is heavily dashed to the south of latitude  $6^\circ\text{N}$  where the oceanographic observations upon which it is based were all taken in October–November, although in different years. To the north of  $6^\circ\text{N}$  the curve is shown by light dashes because observations off the American west coast in March have been combined with observations in mid-ocean in October. The right-hand term, the stress function, is shown by a full-drawn curve and is based on climatological wind data for the months October–November. The agreement between the curves is very good, considering that results of average wind conditions are compared with results derived from a few oceanographic stations which have been occupied in different seasons.

In figure 2 the  $P$  function and the terms of equation (19) are plotted against latitude. The  $P$  function is based on the *Carnegie* observations

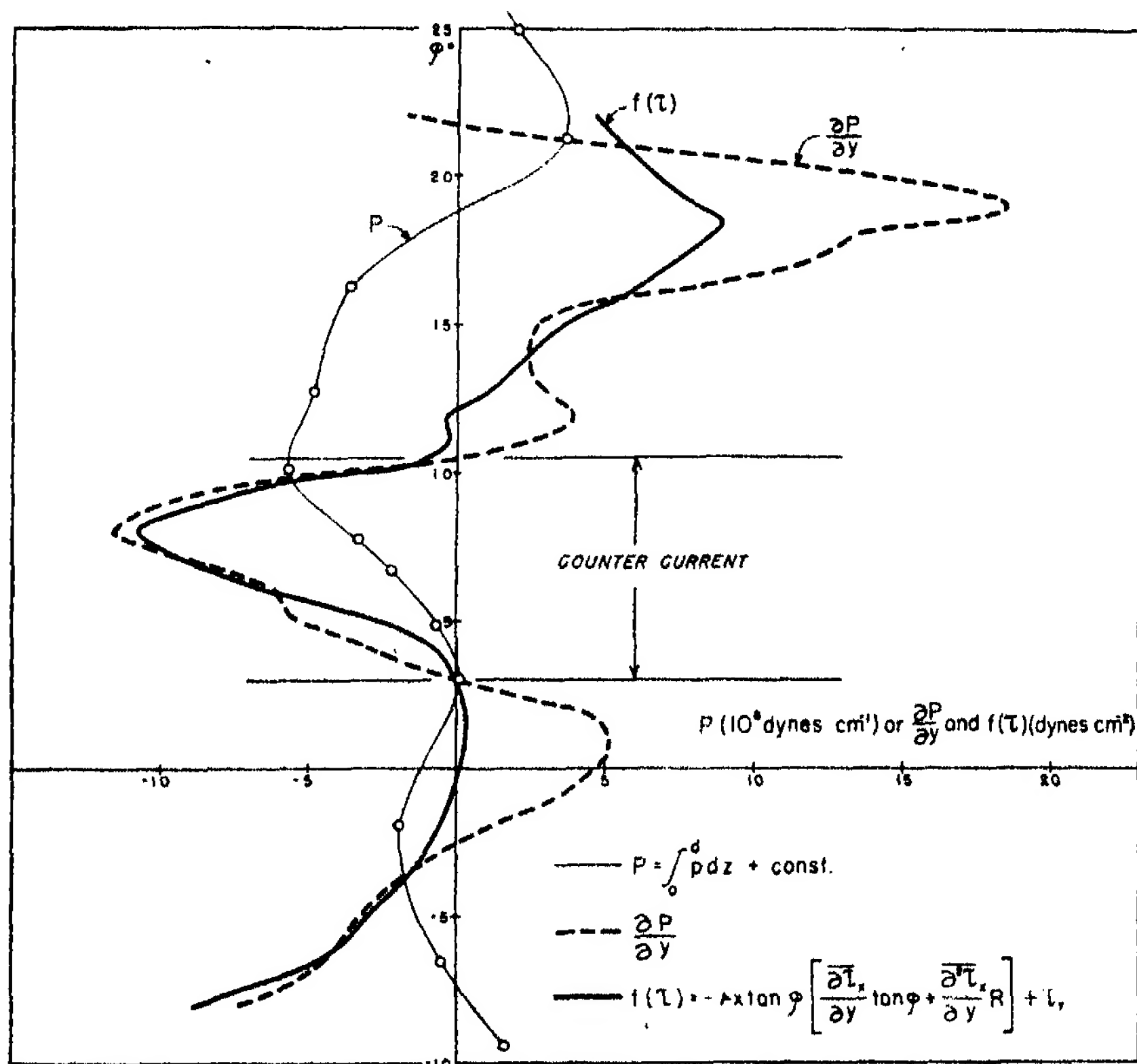


FIGURE 2

in mid-ocean in October–November, 1928, and the stress function on the average wind conditions in October–November over the ocean from the American west coast to the *Carnegie* section. A good agreement is obtained between the results based on a single oceanographic section and those derived from climatological wind charts.

4. *Conclusions.*—The distribution of density and the mass transport by the accompanying currents of the eastern equatorial Pacific depend entirely upon the average stress exerted on the sea surface by the prevailing winds. This conclusion is probably valid for the equatorial currents of all oceans but it has been demonstrated only for a case in which the non-linear terms in the equation of motion can be neglected.

It appears possible that the analysis of the relationship between wind stress and prevailing currents, assuming baroclinic conditions, can be

extended to other cases and can be developed into a powerful tool for examining permanent currents as well as changes produced by changing winds. Efforts in this direction are being continued.

\* Contributions from the Scripps Institution of Oceanography, New Series, No. 324.

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<sup>2</sup> Stockmann, W., *Comptes rendus (Doklady) l'Acad. sci. l'U.R.S.S.*, **52**, 309-312 (1946).

<sup>3</sup> Fjeldstad, J. E., *Archiv Math. Naturvid.*, **48**, no. 6 (1946).

<sup>4</sup> Montgomery, R. B., and Palmén, E., *Jour. Marine Research*, **3**, 112-133 (1940).

<sup>5</sup> Ekman, V. W., *Gerlands Beitr. z. Geophysik*, **Suppl. 4** (1939).

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<sup>7</sup> Fleming, J. A., et al., *Sci. Results Cruise VII "Carnegie" 1928-29*, **I-B** (1945).

<sup>8</sup> Sverdrup, H. U., and Staff, *Records Observations, Scripps Institution of Oceanography*, **1**, 65-160 (1943).

<sup>9</sup> U. S. Weather Bureau, *W. B. No. 1247* (1938).

<sup>10</sup> Rossby, C.-G., *Papers Phys. Oceanography Meteorology*, **4**, no. 3 (1936).

## THE PROBLEMS OF CONGRUENT NUMBERS AND CONCORDANT FORMS

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1. *Four Related Problems.*—All letters in formulas denote rational integers, and solution means the *complete* solution in such integers. The problem of solving the simultaneous diophantine equations

$$rX^2 + mY^2 = rZ^2, \quad sX^2 + nY^2 = sW^2$$

includes as special cases two classical problems.

*Problem 1.*—If  $r = s = Y^2 = 1$ ,  $n = -m$ , where  $m$  is a given constant, the problem is that of congruent numbers. It goes back to Diophantus in the third century, the Arabs of the tenth and eleventh centuries, and Leonardo of Pisa (Fibonacci) in the early thirteenth century. For  $m$  arbitrarily assigned it is still unsolved.

*Problem 2.*—For  $r = s = 1$  the problem is Euler's (1780) of concordant forms, also unsolved.

Many special cases of these two have been investigated. Thus Fermat proved by his method of descent that if  $m = n = -1$  in Problem 2, there are no integers  $X, Y, Z, W$  all different from zero satisfying the equations. From this his theorem for fourth powers follows. Modern work originating in these problems has been concerned with cubics and quartics having

at most a finite number of sets of values of the indeterminates satisfying the equations. Some of this has used the theory of the units in special algebraic number rings. From the results it is possible, by the method applied to Problem 3, to derive much information on new diophantine systems of degrees higher than the second. This will be considered elsewhere. For the present, the inherent complexity of the solution of Problem 3 may suggest why these two old and apparently simple problems are still not completely solved.

*Problem 3.*—To state necessary and sufficient forms of  $r, m, s, n$  in order that there shall exist  $X, Y, Z, W$  all different from zero satisfying the equations.

A special case that has been frequently discussed may be noted. In Problem 1, the required form of  $m$  is given by

$$4m = xyz^2w^2(x^2 - y^2), w(x + y) \text{ even.}$$

The corresponding  $X, Z, W$  are given by

$$4X = zw(x^2 + y^2), 4Z = zw(x^2 + 2xy - y^2), 4W = zw(x^2 - y^2).$$

For  $m$  squarefree,  $zw = \pm 1$ , giving a known criterion. The proof is immediate by the method used for solving Problem 3. Although it is not included in Problem 3, another, somewhat similar problem, dating from the Arabs and usually included with questions on congruent numbers is

*Problem 4.*—To state a necessary and sufficient form of  $n$  in order that  $X, Y, Z$  all different from zero shall exist satisfying

$$n + X^2 = Y^2, n - X^2 = Z^2.$$

The solution is given by

$$4n = x^2(a^2y^4 + b^2z^4), ab = 2;$$

the corresponding  $X, Y, Z$  are given by

$$X = xyz, 2Y = x(ay^2 + bz^2), 2Z = x(ay^2 - bz^2).$$

This is equivalent to

$$ab = 2, fgh^2 = 4, a^2y^4 + b^2z^4 = fu, n = guv^2;$$

$$X = ghyzv, 2Y = ghv(ay^2 + bz^2), 2Z = ghv(ay^2 - bz^2).$$

2. *Solution of Problem 3.*—If  $r, m, s, n, X, Y, Z, W$  are indeterminates, the equations are homogeneous cubics, each of which is separable and hence (completely) solvable. The result of equating the parametric expressions for  $X$  and those for  $Y$  in the solutions gives a separable and hence (completely) solvable system. As the solution of separable equations,



or of a system of such equations, is now straightforward routine, it will suffice to state the final result. To condense the formulas, write

$$\begin{aligned} a &\equiv a_1 a_2 a_3 a_4 a_5, & b &\equiv b_1 b_2 b_3 b_4 b_5, & c &\equiv c_1 c_2 c_3 c_4 c_5, \\ f &\equiv f_1 f_2 f_3 f_4 f_5, & g &\equiv g_1 g_2 g_3 g_4 g_5, & h &\equiv h_1 h_2 h_3 h_4 h_5, \\ \alpha &\equiv b_1 c_1 f_1 g_1 h_1, & \beta &\equiv a_1 c_2 f_2 g_2 h_2, & \gamma &\equiv a_2 b_2 f_3 g_3 h_3, \\ \theta &\equiv a_3 b_3 c_3 g_3 h_4, & \phi &\equiv a_4 b_4 c_4 f_4 h_5, & \psi &\equiv a_5 b_5 c_5 f_5 g_5; \\ \pi &\equiv abc fgh, & m &\equiv pm_1 m_2, & n &\equiv tn_1 n_2. \end{aligned}$$

Thus  $p, m_1, m_2$  are bound parameters whose product is  $m$ ; similarly for  $t, n_1, n_2$  and  $n$ . The  $a_i, \dots, g_i$  are independent parameters. Define

$$\begin{aligned} A &\equiv m_1 a f g^2 - n_1 \alpha \theta \phi^2, & B &\equiv m_2 b f h^2 - n_2 \beta \theta \psi^2, \\ C &\equiv m_2 n_1 b h^2 \alpha \phi^2 - m_1 n_2 a g^2 \beta \psi^2, \end{aligned}$$

introduce the parameters  $x, y, z$  and define  $e$ , for assigned values of all the parameters, as an arbitrary integer multiple of the reciprocal of the greatest common divisor of

$$xy^2 A, \quad xz^2 B, \quad y^2 z^2 C.$$

(If  $e$  is merely an arbitrary integer, the values of  $r, s, X, Y, Z, W$  stated presently, with  $p, m_1, m_2, t, n_1, n_2$  as above, satisfy the equations identically, but this does not exhaust the possibilities. The stated definition of  $e$  is necessary.) Introduce the parameter  $u$ . The required values of  $r, s$  are

$$r = e^2 p u^2 x^2 y^2 z^2 a b c^2 A B, \quad s = e^2 t u^2 x^2 y^2 z^2 \alpha \beta \gamma^2 A B.$$

To state the corresponding values of  $X, Y, Z, W$  define

$$\begin{aligned} F &\equiv m_2 n_1 b h^2 \alpha \phi^2 - a g^2 \beta \psi^2 \\ G &\equiv 2 m_1 m_2 a b f g^2 h^2 - m_1 n_2 a g^2 \beta \theta \psi^2 - m_2 n_1 b h^2 \alpha \theta \phi^2, \\ H &\equiv m_1 n_2 a f g^2 \beta \psi^2 + m_2 n_1 b f h^2 \alpha \phi^2 - 2 n_1 n_2 \alpha \beta \theta \phi^2 \psi^2. \end{aligned}$$

Introduce a parameter  $k$ . Then

$$\begin{aligned} 2X &= e k x^2 y^2 z^2 f \theta F, & Y &= e^3 k u x^2 y^2 z^2 \pi A B, \\ 2Z &= e k x^2 y^2 z^2 f G, & 2W &= e k x^2 y^2 z^2 \theta H. \end{aligned}$$

Including the bound parameters  $p, m_1, m_2, t, n_1, n_2$  there are in all 41. Each of  $m, n$  is of degree 3; each of  $r, s$ , is of degree 71; each of  $X, Z, W$  is of degree 49, and  $Y$  is of degree 83. The degree of each of the identities giving the solution of the cubic system is thus 169.

## ON A THEOREM OF VON NEUMANN

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In these PROCEEDINGS, August 15, 1946, Loomis<sup>1</sup> presented a simple proof of a theorem due to J. von Neumann<sup>2, 3</sup> and formulated by Loomis as follows:

Let  $a_{ij}$  and  $b_{ij}$  be two rectangular matrices ( $i = 1, \dots, n$ ), ( $j = 1, \dots, m$ ) such that  $a_{ij} > 0$  for all  $i, j$ . Then there exists a unique  $\lambda$  and vectors  $x = (x_1, \dots, x_m)$ ,  $y = (y_1, \dots, y_n)$  subject to  $x_j \geq 0$ ,  $y_i \geq 0$ ,  $\sum_1^m x_j = 1$  and  $\sum_1^n y_i = 1$  such that

$$\lambda \sum_{j=1}^m a_{ij} x_j \geq \sum_{j=1}^m b_{ij} x_j, \quad i = 1, \dots, n, \quad (1)$$

$$\lambda \sum_{i=1}^n a_{ij} y_i \leq \sum_{i=1}^n b_{ij} y_i, \quad j = 1, \dots, m. \quad (2)$$

The present note gives an alternative simple proof, the essence of which is a reduction of the question to consideration of a single system of linear equations. This reduction is accomplished in two steps: 1st, replacement of (1) and (2) by an equivalent pair of adjoint linear systems

$$\sum_{j=1}^m \alpha_{ij} x_j \geq 0, \quad i = 1, \dots, n + m, \quad (1')$$

$$\sum_{i=1}^{n+m} \alpha_{ij} y_i = 0, \quad j = 1, \dots, m; \quad (2')$$

and 2nd, utilization of a known relationship<sup>4</sup> between adjoint systems of form (1'), (2'). This relationship is simply that *the system of inequalities (1') admits a solution  $x$  which does not annul all the left members if, and only if, the system of equations (2') admits no positive solution  $y$  (every  $y_i$  positive).*

The system (1') consists of  $n + m$  inequalities, the first  $n$  of which are those in (1) after obvious transpositions, and the last  $m$  are the conditions  $x_j \geq 0$ ,  $j = 1, \dots, m$ , which occur as collateral conditions in the statement of the theorem.

The system (2') consists of  $m$  equations, which are equivalent to the  $m$  inequalities of (2) by virtue of the introduction of  $m$  new and essentially non-negative variables  $y_{n+1}, \dots, y_{n+m}$ .

The explicit definitions of the coefficients  $\alpha_{ij}$  are

$$\begin{aligned} \alpha_{ij} &= \lambda a_{ij} - b_{ij}, & i &= 1, \dots, n; & j &= 1, \dots, m, \\ \alpha_{ij} &= \delta_{ij}, & i &= n + 1, \dots, n + m; & j &= 1, \dots, m, \end{aligned}$$

where  $\delta_{ij} = 1$  if  $i = n + j$ ; otherwise it is zero.

In view of the relationships which have been indicated above, the von Neumann theorem will be justified if we show the existence of a unique value of the parameter  $\lambda$  for which the system of equations (2') admits no *positive* solution  $y = (y_1, \dots, y_{n+m})$ , but does admit a *non-negative* solution with at least one  $y_k$  positive.

To this end, we consider the explicit form of the system (2'), which may be written

$$\sum_{i=1}^n (\lambda a_{ij} - b_{ij}) y_i + y_{n+j} = 0, \quad j = 1, \dots, m. \quad (2'')$$

Since  $a_{ij} > 0$  for every  $i, j$ , it is obvious that for  $\lambda$  sufficiently small the system will admit positive solutions  $y$ , while for  $\lambda$  sufficiently large it will admit no such solution. Furthermore if it admits a positive solution for any particular value of  $\lambda$ , it will admit such a solution for any smaller value. We denote by  $\lambda_0$  the least upper bound of the values of  $\lambda$  for which (2'') admits a positive solution  $y$ . Since to every positive solution  $y$  there corresponds by simple normalization a solution for which  $\sum_1^{n+m} y_k = 1$ , it follows from the compactness of the closed and bounded sub-space defined by  $y_k \geq 0$ ,  $\sum_1^{n+m} y_k = 1$  that to the value  $\lambda = \lambda_0$  there corresponds a solution of (2'') in this subspace; call it  $y^0 = (y_1^0, \dots, y_{n+m}^0)$ .

At least one of the coördinates  $y_k^0$  must be *zero*. For if they were all positive, a sufficiently small  $\epsilon > 0$  would make correspond to the parameter value  $\lambda_0 + \epsilon$  a *positive* solution  $y' = (y_1', \dots, y_{n+m}')$  with  $y_i' = y_i^0$ , ( $i = 1, \dots, n$ ), and  $y_{n+j}' = y_{n+j}^0 - \epsilon \sum_1^n a_{ij} y_i^0$ , ( $j = 1, \dots, m$ ), thus contradicting the definition of  $\lambda_0$ . In  $\lambda_0$  we have therefore *one* value of the parameter  $\lambda$  which affords an admissible solution.

No second value of  $\lambda$  does. For if  $\lambda_1 > \lambda_0$  did afford an admissible solution  $y'$ , then  $\sum_1^n (\lambda_1 a_{ij} - b_{ij}) y_i' \leq 0$ , ( $j = 1, \dots, m$ ), and consequently, for any  $\epsilon > 0$

$$\sum_{i=1}^n [(\lambda_1 - \epsilon) a_{ij} - b_{ij}] y_i' < 0, \quad j = 1, \dots, m,$$

and from the continuity of the linear functions these inequalities remain strictly valid if each  $y_i'$  be replaced by  $y_i'' = y_i' + \eta_i$  with  $\eta_i > 0$  and sufficiently small. Defining  $y_{n+j}''$ , ( $j = 1, \dots, m$ ) by the equations

$$\sum_{i=1}^n [(\lambda_1 - \epsilon) a_{ij} - b_{ij}] y_i'' + y_{n+j}'' = 0, \quad j = 1, \dots, m,$$

we would then have a *positive* solution corresponding to the parameter value  $\lambda_1 - \epsilon > \lambda_0$ , thus contradicting the definition of  $\lambda_0$ .

This completes the proof of the theorem. The method of proof definitely suggests the possibility of generalization, since the vital relationship between the adjoint systems (1') and (2') persists when the finite matrix

$\alpha_{ij}$  is replaced by a function  $\alpha(p, q)$  and the summations are replaced by more general linear operators.

<sup>1</sup> Loomis, L. H., "On a Theorem of von Neumann," *Proc. Nat. Acad. Sci.*, **32**, 213-215 (1946).

<sup>2</sup> von Neumann, J., "Über ein ökonomisches Gleichungssystem, etc.," *Ergebnisse eines Mathematischen Kolloquiums*, **8**, 73-83 (1937).

<sup>3</sup> von Neumann, J., and Morgenstern, O., "*Theory of Games and Economic Behavior*," Princeton University Press (1944).

<sup>4</sup> Dines, L. L., "Convex Extension and Linear Inequalities," *Bull. Amer. Math. Soc.*, **42**, 353-365 (1936).

## NOTE ON CONTINUOUS REPRESENTATIONS OF LIE GROUPS

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Let  $G$  be an analytic Lie group with unit element  $e$  and let  $a \rightarrow T(a)$ , ( $a \in G$ ), be a representation of  $G$  as bounded operators  $T(a)$  on a Banach space  $B$  such that for every  $x \in B$ ,  $T(e)x = x$  and  $T(a)x \rightarrow T(b)x$  when  $a \rightarrow b$ . Let  $A = (a(s))$ , ( $s$  real), be a one-parameter subgroup in  $G$  and define

$$T_A x = \lim_{s \rightarrow 0} s^{-1}(T(a(s)) - 1)x$$

whenever the limit exists in which case  $x$  is said to be in the domain of  $T_A$ . I. Gelfand proved that every such domain is dense in  $B$ .<sup>1</sup> By a slight extension of his method we will prove that they have a dense intersection when  $A$  varies, thus answering a question left open by W. Wigner<sup>2</sup> and V. Bargmann.<sup>3</sup>

Let  $r$  be a not-negative integer or  $+\infty$ , let  $C_r$  be the class of real functions defined on  $G$  with continuous derivatives of order  $\leq r$ , ( $< r$  if  $r = \infty$ ), and let  $C_r^0$  be the subset of  $C_r$  whose elements vanish outside a compact set and let  $B_r$  be the set of elements of  $B$  of the form

$$x(h) = \int_G h(b)T(b)x db \quad (h \in C_r^0, x \in B),$$

where  $db$  is a left invariant volume element on  $G$ .

**THEOREM.** Every  $B_{r+1}$  is dense in  $B$ , it is in the domain of any  $T_A$  and  $T_A B_{r+1}$  is contained in  $B_r$  for any  $A$ .

*Proof:* One has with  $s \neq 0$

$$\begin{aligned} s^{-1}(T(a(s)) - 1)x(h) &= s^{-1} \int_G h(b)(T(a(s)b) - T(b))x db = \\ &= \int_G s^{-1}(h(a^{-1}(s)b) - h(b))T(b)x db. \end{aligned}$$

Hence, when  $s \rightarrow 0$

$$T_A x(h) = x(h_A),$$

where

$$h_A(b) = \lim_{s \rightarrow 0} s^{-1}(h(a^{-1}(s)b) - h(b)) \in C_r^0$$

if  $h \in C_{r+1}^0$ . This proves the second part of the theorem. To prove the first part we remark that it is possible to choose a sequence of not-negative functions  $h_1, h_2, \dots$  in  $C_\infty^0$  such that  $\int_G h_k(a) da = 1$  and for any open set  $C$  containing  $e$ ,  $\int_{G-C} h_k(a) da = 0$  when  $k$  is sufficiently large. Then  $x(h_k) \in B_r$  and  $x(h_k) \rightarrow x$  when  $k \rightarrow \infty$  for any  $x$  and  $r$ .

Similar theorems can be obtained for semigroups with a unit element and even for differentiable manifolds as follows.

Let  $M$  be a  $C_2$ -manifold of  $m$  dimensions in the sense of Whitney<sup>4</sup> with defining mappings  $\theta_1, \theta_2, \dots$  of the interior  $Q$  of the unit  $(m-1)$ -sphere into  $M$ . Let  $(p, q) \rightarrow r = f(p, q)$  be a mapping of class  $C_1$  of  $M \times M$  into  $M$  which is locally one-to-one when  $p$  and  $q$  are fixed ( $p, q, r \in M$ ).

Let  $p \rightarrow T(p)$  be a mapping of  $M$  upon a set of bounded operators  $T(p)$  on a Banach space  $B$  which is continuous in the sense used above and is such that there exists a sequence of points  $p_1, p_2, \dots$  in  $M$  such that  $T(p_k)x \rightarrow x$  for every  $x$  in  $B$  when  $k \rightarrow \infty$ , and

$$T(p)T(q) = T(f(p, q)).$$

Let  $p = \theta_k(a)$ , ( $a \in Q$ ), and  $0 \neq b \in Q$  and put

$$T_k(p, b)x = \lim_{s \rightarrow 0} s^{-1}(T(\theta_k(a + sb)) - T(\theta_k(a)))x$$

whenever the limit exists. Then the domains of  $T_k(p, b)$  have a dense intersection  $I$ . In fact it is easily verified that the elements of  $B$  of the form

$$x_j(h) = \int_Q h(t) T(\theta_j(t)) x dt \quad (x \in B),$$

belong to  $I$  if  $h(t)$  is of class  $C_1$  and vanishes outside a compact subset of  $Q$ .

To prove that  $I$  is dense in  $B$  it suffices to take a sequence  $j_1, j_2, \dots$  such that  $p_k \in \theta_{j_k}(Q)$  and suitable not-negative functions  $h_1, h_2, \dots$  in  $C_1$  which vanish outside suitable open sets containing  $\theta_{j_k}^{-1}p_k$  so that  $x_{j_k}(h_k) \rightarrow x$  as  $k \rightarrow \infty$ .

<sup>1</sup> Gelfand, I., "On One-Parametrical Groups of Operators in a Normed Space," *C. R. (Doklady) Acad. Sci. URSS*, **25**, 713-718 (1939).

<sup>2</sup> Wigner, E., "On Unitary Representations of the Inhomogeneous Lorentz Group," *Annals of Math.*, **40**, 149-204 (1939).

<sup>3</sup> Bargmann, V., "Irreducible Representations of the Lorentz Group," *Ibid.*, **48**, 568-640 (1947).

<sup>4</sup> Whitney, H., "Differentiable Manifolds," *Ibid.*, **37**, 645 (1936).

## ON LACUNARY TRIGONOMETRIC SERIES

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Let us consider a lacunary trigonometric series

$$\sum_{k=1}^{\infty} (a_k \cos n_k x + b_k \sin n_k x), \text{ with } n_{k+1}/n_k > q > 1, \quad (1)$$

and let us confine our attention to the interval  $0 \leq x \leq 2\pi$ . Let  $S_N(x)$  denote the  $N$ th partial sum of (1), that is to say the sum of the terms with  $k = 1, 2, \dots, N$ . Let  $C_N = \{1/2(a_1^2 + b_1^2 + \dots + a_N^2 + b_N^2)\}^{1/2}$ . In a recent note,<sup>1</sup> J. Ferrand and R. Fortet considered the behavior of the ratio  $S_N(x)/C_N$ . They stated, without proof, that if  $C_N \rightarrow +\infty$  as  $N \rightarrow \infty$ , the distribution function of  $S_N(x)/C_N$  tends to the Gaussian distribution with mean value 0 and dispersion 1. Clearly, the result as stated cannot be correct since, if  $c_k = (a_k^2 + b_k^2)^{1/2}$  increases very rapidly, the distribution function tends to that of cosine. Thus some kind of restriction on the  $c_k$  is necessary. (Mr. Erdős informs us that a proof of the result, under the condition  $C_n \rightarrow \infty$ ,  $c_n = O(1)$ , will be published in a paper written jointly by him, Mlle. Ferrand, Fortet and Kac.) In this note we propose to give a complete solution of the problem.

By a distribution function we shall mean any non-decreasing and continuous to the right function  $F(y)$  with  $F(-\infty) = 0$ ,  $F(+\infty) = 1$ . Let  $Z_n(y)$  be the set of points  $x$  from  $(0, 2\pi)$  at which  $S_N(x)/C_N \leq y$ . Let  $F_N(y) = |Z_N(y)|/2\pi$  (by  $|E|$  we mean the measure of the set  $E$ ), so that  $F_N$  is the distribution function of  $S_N/C_N$ .

(i) *If  $F_N(y)$  tends to a distribution function  $F(y)$  (at the points of continuity of the latter) such that either  $F(y) > 0$  or  $F(y) < 1$  for all finite  $y$ , then*

$$c_n/C_n \rightarrow 0. \quad (2)$$

(ii) *If (2) is satisfied and if  $C_n \rightarrow \infty$ , then  $F_N(y)$  tends to the Gaussian distribution with mean value 0 and dispersion 1.*

We shall even prove a more general result, namely, that the distribution function of  $S_N(x)/C_N$  on every fixed set of positive measure tends to the Gaussian distribution.

(iii) *Let  $E$  be a point set on  $(0, 2\pi)$ , with  $|E| > 0$ , and let  $F_N(y; E) = |Z_N(y) \cap E|/|E|$ . If  $C_n \rightarrow \infty$  and if (2) holds, then  $F_N(y; E)$  tends to the Gaussian distribution with mean value 0 and dispersion 1.*

To prove (i), let us assume for example that  $F(y) < 1$  for all finite  $y$ , and that (2) is false. Hence  $c_N/C_N > \sqrt{2}\epsilon > 0$  for infinitely many  $N$ .



Let us confine our attention to such  $N$ . Obviously,  $C_{N-1}/C_N < (1 - \epsilon^2)^{1/2}$ , and the formula,

$$\frac{S_N}{C_N} = \frac{S_{N-1}}{C_{N-1}} \cdot \frac{C_{N-1}}{C_N} + \frac{a_N \cos n_N x + b_N \sin n_N x}{C_N},$$

shows that at every point  $x$  from  $Z_{N-1}(y)$ ,  $y > 0$ , we have  $S_N(x)/C_N \leq y(1 - \epsilon^2)^{1/2} + \sqrt{2}$ . It follows that  $Z_{N-1}(y)$  is included in  $Z_N(y(1 - \epsilon^2)^{1/2} + \sqrt{2})$ , and so  $F_{N-1}(y) \leq F_N(y(1 - \epsilon^2)^{1/2} + \sqrt{2})$ . Let  $y$  be a point of continuity of  $F$ . Making  $N \rightarrow \infty$ , we get  $F(y) \leq F(y(1 - \epsilon^2)^{1/2} + \sqrt{2})$ . However, from the assumption that  $F(y)$  is always  $< 1$  we obtain that there are points  $y > 0$  of continuity of  $F$  at which  $F(y(1 - \epsilon^2)^{1/2} + \sqrt{2}) < F(y)$ . This contradiction completes the proof of (i). Condition  $C_N \rightarrow \infty$  and condition (2) imply that  $\text{Max}_{1 \leq k \leq N} c_k / C_N \rightarrow 0$  as  $N \rightarrow \infty$ . This remark will be used repeatedly below.

To prove (iii), let us assume for the sake of brevity that (1) is a purely cosine series, and let us write  $A_N$  for  $C_N$  (in the general case we merely represent the terms of (1) in the form  $c_k \cos(n_k x + \theta_k)$  and the argument that follows remains unchanged). It is enough to prove that over any finite range of  $\lambda$  the characteristic function of  $F_N(y; E)$  uniformly approaches that of the Gaussian distribution. The characteristic function of  $F_N(y; E)$  is

$$\begin{aligned} \int_{-\infty}^{+\infty} e^{i\lambda y} dF_N(y; E) &= |E|^{-1} \int_E e^{i\lambda S_N(x)/A_N} dx = \\ &= |E|^{-1} \int_E \exp. \left\{ i\lambda A_N^{-1} \sum_{k=1}^N a_k \cos n_k x \right\} dx = \\ &= |E|^{-1} \int_E e^{o(1)} \prod_{k=1}^N \left( 1 + \frac{i\lambda a_k}{A_N} \cos n_k x \right) \exp. \left\{ -\frac{1}{2} \frac{\lambda^2 a_k^2}{A_N^2} \cos^2 n_k x \right\} dx, \quad (3) \end{aligned}$$

on account of (2) and of the relation  $\exp. z = (1 + z) \exp. \{ \frac{1}{2} z^2 + o(|z|^2) \}$  valid for  $z \rightarrow 0$ . Due to (2) and  $A_N \rightarrow \infty$ , the term  $o(1)$  in  $e^{o(1)}$  tends to 0 uniformly in  $x$  as  $N \rightarrow \infty$  (provided  $\lambda = O(1)$ , an assumption we always make from now on). Let us now observe that

$$\left| \prod_{k=1}^N \left( 1 + \frac{i\lambda a_k}{A_N} \cos n_k x \right) \right| \leq \prod_{k=1}^N \left( 1 + \frac{\lambda^2 a_k^2}{A_N^2} \right)^{1/2} \leq e^{\gamma^2}, \quad (4)$$

and that

$$\sum_{k=1}^N \frac{a_k^2}{A_N^2} \cos^2 n_k x = 1 + \sum_{k=1}^N \frac{a_k^2}{2A_N^2} \cos 2n_k x = 1 + \xi_N(x).$$

The measure of the set of points where  $|\xi_N(x)| \geq \delta > 0$  is less than  $\delta^{-2} \int_0^{2\pi} \xi_N^2(x) dx = \frac{1}{4\pi\delta^2} (a_1^4 + \dots + a_N^4) / A_N^4$ , and so tends to 0 on account of (2) and  $A_N \rightarrow \infty$ . Since  $|\xi_N(x)| \leq 1$ , it follows that, with an error tending uniformly to 0 as  $N \rightarrow \infty$ , the integral (3) is

$$|E|^{-1} e^{-\lambda^2/2} \int_E \prod_{k=1}^N \left( 1 + \frac{i\lambda a_k}{A_N} \cos n_k x \right) dx = |E|^{-1} e^{-\lambda^2/2} I_N, \quad (5)$$

and it is enough to prove that  $I_N$  tends uniformly to  $|E|$ . We shall write

$$\prod_1^N \left( 1 + i\lambda \frac{a_k}{A_N} \cos n_k x \right) = \alpha_0^{(N)} + \sum_1 \alpha_\gamma^{(N)} \cos \gamma x, \quad \epsilon_k = \epsilon_k^{(N)} = |i\lambda a_k/A_N|, \quad (6)$$

so that  $\alpha_\gamma^{(N)}, \epsilon_k$  depend on  $\lambda$  also.

If  $q \geq 3$ , the proof of  $I_N \rightarrow |E|$  is simple. For the numbers  $\alpha_\gamma^{(N)}$  which actually occur in (6) correspond to the indices  $\gamma \geq 0$  of the form  $\pm n_{k_1} \pm n_{k_2} \pm \dots \pm n_{k_p}$  ( $k_1 > k_2 > \dots > k_p$ ). For  $q \geq 3$  such a representation (if it exists) is unique. In particular,  $\alpha_0^{(N)} = 1$ . Thus  $I_N = |E| + \pi \sum h_\gamma \alpha_\gamma^{(N)}$ , where  $h_\gamma$  are the cosine Fourier coefficients of the characteristic function of  $E$ . On account of (2), each  $\alpha_\gamma^{(N)}$  tends to 0 as  $N \rightarrow \infty$  and  $\gamma = 1, 2, \dots$ . Thus, if  $\gamma_0$  is fixed,

$$\sum_1 |h_\gamma \alpha_\gamma^{(N)}| \leq \sum_1^{\gamma_0} + \sum_{\gamma_0+1} \leq o(1) + \left( \sum_{\gamma_0+1}^\infty h_\gamma^2 \right)^{1/2} \left( \sum_{\gamma_0+1} |\alpha_\gamma^{(N)}|^2 \right)^{1/2}. \quad (7)$$

The first factor in the last product is arbitrarily small if  $\gamma_0$  is large enough (since  $\sum h_\gamma^2 < \infty$ ), and the last factor is bounded by, (6), (4), and Parseval's formula. Thus  $I_N \rightarrow |E|$ , and (iii) follows. (If  $E = (0, 2\pi)$ , then even the weaker condition  $q \geq 2$  implies that  $\alpha_0^{(N)} = 1$ , so that  $I_N = 2\pi$ , and (ii) follows.)

The proof of the general case of (iii) is based on two lemmas.

LEMMA 1. *The number of solutions of  $n_{k_1} + n_{k_2} + \dots + n_{k_p} = a$ , where  $a$  is given is less than  $C^{p-1}$ , where  $C = C(q)$  depends on  $q$  only. For assuming that  $k_1 > k_2 > \dots > k_p$ , we have  $n_{k_1} \leq a \leq n_{k_1} q/(q-1)$ , so that  $n_{k_1}$  takes  $\leq C(q)$  values. The same applies to  $n_{k_2}$  when  $n_{k_1}$  is fixed and so on.*

LEMMA 2. *The number of solutions of  $n_{k_1} + \dots + n_{k_p} - n_{k_{p+1}} = a$  ( $k_1 > k_2 > \dots > k_p > k_{p+1}$ ) is less than  $C^p$ , where  $C$  is the same as in Lemma 1. For  $p = 1$  this is immediate since  $n_{k_1} - n_{k_2} = a$  implies  $n_{k_1}(1 - 1/q) \leq a \leq n_{k_1}$ , and the ratio of the extreme terms here is the same as in the inequality for  $a$  in the proof of Lemma 1. Combining this special case with Lemma 1 we get Lemma 2.*

Let us now revert to the first formula (6). As the argument (7) shows, (iii) will be established when we show that  $\alpha_0^{(N)} \rightarrow 1$ ,  $\alpha_\gamma^{(N)} \rightarrow 0$  for  $\gamma = 1, 2, \dots$ . As a matter of fact, we can prove that  $\alpha_\gamma^{(N)} \rightarrow 0$  uniformly in  $\gamma > 0$ . The  $\gamma$  actually occurring in (6) are  $\geq 0$  and of the form  $\pm n_{k_1} \pm n_{k_2} \pm \dots \pm n_{k_p}$  ( $k_1 > k_2 > \dots > k_p$ ). Let us fix  $p$  and let us write the last equation in the form

$$n_{k_1} + n_{k_2} + \dots + n_{k_p} = \gamma + n_{k_1} + n_{k_2} + \dots + n_{k_p} \quad (8)$$

with  $p = s + t$ ,  $h_1 > h_2 > \dots > h_s$ ,  $l_1 > l_2 > \dots > l_t$ . Let us also fix  $s$  and  $t$ . Clearly, the case  $s = t = 0$  leads to the contribution 1 in  $\alpha_0^{(N)}$  so that, in order to estimate  $\alpha_0^{(N)} - 1$ ,  $\alpha_\gamma^{(N)}$  ( $\gamma = 1, 2, \dots$ ) we must assume that  $s \geq 1$ ,  $t \geq 0$ . For the clarity of presentation, let us first assume that  $s > 1$ ,  $t > 1$ . Let us also assume that  $h_s < l_t$ . Thus we investigate the solutions of (8) under the conditions

$$\gamma \geq 0, s > 1, t > 1 - \text{fixed}, h_s < l_t. \quad (9)$$

Every solution of (8) contributes to  $\alpha_\gamma^{(N)}$  (or to  $\alpha_0^{(N)} - 1$ ) as much as  $\epsilon_{h_1} \dots \epsilon_{h_s} \epsilon_{l_1} \dots \epsilon_{l_t} / 2^{p-1}$  in absolute value. The total contribution of the solutions of (8) satisfying (9) is therefore  $\leq S = 2^{-(p-1)} \sum \epsilon_{h_1} \dots \epsilon_{h_s} \epsilon_{l_1} \dots \epsilon_{l_t}$ , summation being extended over all indices satisfying (8) and (9). Let  $\eta = \max. (\epsilon_1, \epsilon_2, \dots, \epsilon_N)$  so that  $\eta = \eta_{N,\lambda} \rightarrow 0$  as  $N \rightarrow \infty$ ,  $\lambda = O(1)$ . Then  $S \leq 2^{-(p-1)} \eta \sum \epsilon_{h_1} \dots \epsilon_{h_{s-1}} \epsilon_{l_1} \dots \epsilon_{l_t}$  where summation is extended over the same indices  $h_1, \dots, h_s, l_1, \dots, l_t$  as before. It follows that

$$S \leq 2^{-(p-1)} \eta (\sum \epsilon_{h_1}^2 \dots \epsilon_{h_{s-1}}^2)^{1/2} (\sum \epsilon_{l_1}^2 \dots \epsilon_{l_t}^2)^{1/2} \quad (10)$$

In  $\sum \epsilon_{l_1}^2 \dots \epsilon_{l_t}^2$  each term must be taken as many times as for given  $l_1, l_2, \dots, l_t$  one can find various groups of  $h_1, h_2, \dots, h_s$  satisfying (8). By (8) and Lemma 1, this sum is  $\leq C^{s-1} \sum^* \epsilon_{l_1}^2 \dots \epsilon_{l_t}^2$ , the star meaning that now each term occurs once only. Since  $\sum^* \epsilon_{l_1}^2 \dots \epsilon_{l_t}^2 \leq (\epsilon_1^2 + \dots + \epsilon_N^2)^t / t! = (2\lambda^2)^t / t!$ , we get  $\sum \epsilon_{l_1}^2 \dots \epsilon_{l_t}^2 \leq 2^t C^{s-1} \lambda^{2t} / t!$ . Similarly, in the sum  $\sum \epsilon_{h_1}^2 \dots \epsilon_{h_{s-1}}^2$  in (10) each term must be taken as many times as for given  $h_1, h_2, \dots, h_{s-1}$  one can find various groups of  $l_1, \dots, l_t$  satisfying (8). Since  $h_s < l_t$ , an application of Lemma 2 gives

$$\begin{aligned} \sum \epsilon_{h_1}^2 \dots \epsilon_{h_{s-1}}^2 &\leq C^t \sum^* \epsilon_{h_1}^2 \dots \epsilon_{h_{s-1}}^2 = (\epsilon_1^2 + \dots + \epsilon_N^2)^{s-1} C^t / (s-1)! \\ &= C^t (2\lambda^2)^{s-1} / (s-1)! \end{aligned}$$

Hence

$$S \leq \eta (\lambda^2 C / 2)^{(p-1)/2} / \{(s-1)t!\}^{1/2} \leq \eta (\lambda^2 C / 2)^{(p-1)/2} / \{[1/2(p-1)]!\}^{1/2}.$$

If we drop the last condition in (9), we have to double this estimate. If we vary  $s, t$  but keep  $p = s + t$  fixed, the result will be less than  $p$  times the last expression. Finally, summing over  $p$  and observing that the series with terms  $(\lambda^2 C / 2)^{(p-1)/2} p / \{[1/2(p-1)]!\}^{1/2}$  is uniformly convergent in  $\lambda = O(1)$ , we see that the total contribution of the terms so far considered is  $\leq H\eta$ , where  $H$  is uniformly bounded for  $\lambda = O(1)$ . If we obtain a similar estimate for the terms so far disregarded, (iii) will be established.

But  $s = 0$  implies  $t = 0$  and can be omitted. If  $t = 0$ , we argue as before (by majorizing  $\epsilon_{h_s}$  by  $\eta$ ). The case  $s = 1$  with  $h_1 < l_t$  leads to  $\gamma + n_{h_1} + \dots + n_{l_t} - n_{h_s} = 0$  and is impossible; if  $l_t < h_1$ , the treatment is

as above. Finally, if  $t = 1$  ( $s > 1$ ), and  $n_{h_1} + \dots + n_{h_s} = \gamma + n_{h_1}$ , the case  $h_s < l_1$  offers nothing new; if  $h_s > l_1$ , then  $S \leq \eta 2^{-(p-1)} \sum \epsilon_{h_1} \dots \epsilon_{h_s} \leq \eta 2^{-(p-1)} \{C^s \sum^* \epsilon_{h_1}^2 \dots \epsilon_{h_s}^2\}^{1/2}$ , and the argument concludes as before. Thus (iii) is proved.

Suppose now that (1) is of the class  $L^2$ . The remainder  $(a_N \cos n_N x + b_N \sin n_N x) + \dots$  of (1) represents then a certain function  $R_N(x)$ . By  $G_N(y)$  we shall mean the distribution function of  $R_N(x)/D_N$ , where  $D_N = \{1/2(a_N^2 + b_N^2) + \dots\}^{1/2}$ . The proofs of the following two results are repetitions of those of (i) and (iii).

(iv) If (1) is of the class  $L^2$ , and if  $G_N(y)$  tends to the distribution function  $G(y)$  such that either  $G(y) > 0$  or  $G(y) < 1$  for all finite  $y$ , then  $c_N/D_N \rightarrow 0$ .

(v) Suppose that (1) is of the class  $L^2$ , that  $c_N/D_N \rightarrow 0$ , and let  $E$  be a fixed set of positive measure. The distribution function of  $R_N(x)/D_N$  relative to  $E$  tends to the Gaussian distribution with mean value 0 and dispersion 1.

Theorems (iii) and (v) have analogs for power series (2)

$$\sum_{k=1}^{\infty} c_k e^{i n_k x} \quad (n_{k+1}/n_k > q > 1) \quad (11)$$

We shall write  $C_N = \{1/2(|c_1|^2 + \dots + |c_N|^2)\}^{1/2}$ ,  $D_N = \{1/2(|c_N|^2 + \dots)\}^{1/2}$ . The real and the imaginary part of the  $N$ th partial sum of (11) will be denoted by  $U_N(x)$ ,  $V_N(x)$ , and of the  $N$ th remainder of (11) (if  $C_N = O(1)$ ) by  $U'_N(x)$ ,  $V'_N(x)$ , respectively.

(vi) Let  $E$  be a fixed set in  $(0, 2\pi)$  of positive measure. If  $C_N \rightarrow \infty$ ,  $c_N = o(C_N)$ , then the two dimensional distribution function, relative to  $E$ , of the pair of functions  $U_N(x)/C_N$ ,  $V_N(x)/C_N$  tends to  $(2\pi)^{-1} \int_{-\infty}^{\xi} \int_{-\infty}^{\eta} e^{-1/2(\lambda^2 + \mu^2)} d\lambda d\mu$ . The same conclusion holds for  $U'_N(x)/D_N$ ,  $V'_N(x)/D_N$  if (1) is of the class  $L^2$  and if  $c_N = o(D_N)$ .

It is enough to sketch the proof of the first part of (vi). Let  $Z_N(\xi, \eta)$  denote the set of points  $x$  such that  $U_N(x)/C_N \leq \xi$ ,  $V_N(x)/C_N \leq \eta$ , and let  $F_N(\xi, \eta) = |Z_N(\xi, \eta)E|/|E|$ . Let  $c_k = |c_k|e^{i\alpha_k}$ . The characteristic function of  $F_N$  is

$$\begin{aligned} \int_{-\infty}^{+\infty} \int_{-\infty}^{+\infty} e^{i(\lambda\xi + \mu\eta)} dF_N(\xi, \eta) &= |E|^{-1} \int_E e^{i(\lambda U_N + \mu V_N)/C_N} dx = \\ &= |E|^{-1} \int_E \exp. i C_N^{-1} \left\{ \sum |c_k| (\lambda \cos(n_k x + \alpha_k) + \mu \sin(n_k x + \alpha_k)) \right\} dx = \\ &= |E|^{-1} \int_E \exp. \{i C_N^{-1} (\lambda^2 + \mu^2)^{1/2} \sum |c_k| \cos(n_k x + \alpha'_k)\} dx \end{aligned}$$

where the  $\alpha'_k$  depend now on  $\lambda, \mu$ . As  $N \rightarrow \infty$ , and with an error  $o(1)$ , the last expression is

$$\begin{aligned} e^{-1/2(\lambda^2 + \mu^2)} |E|^{-1} \int_E \prod_{k=1}^N \{1 + i C_N^{-1} (\lambda^2 + \mu^2)^{1/2} |c_k| \cos(n_k x + \alpha'_k)\} dx \\ \rightarrow e^{-1/2(\lambda^2 + \mu^2)} \end{aligned}$$

which completes the proof.

The main idea used throughout this paper can be applied to other cases,

for example to linear combinations of the partial sums (or the remainders) of (1) and (11). We can also study the distribution functions in  $(-\infty, +\infty)$  of the partial sums and of the remainders of the series  $\sum c_k e^{i p_k x}$  with linearly independent exponents. We obtain the results parallel to the ones given above but with easier proofs.<sup>3</sup> The infinite products  $\prod(1 + i\alpha_k \cos n_k x)$  can also be used to obtain a simple proof of the theorem of Banach asserting the existence of continuous functions with prescribed Fourier coefficients at lacunary places. To these and other problems we shall return elsewhere.

<sup>1</sup> *Comptes Rendus Paris Acad. Sci.*, 224, 516–878 (1947).

<sup>2</sup> See also *loc. cit.* (1).

<sup>3</sup> See also Kac and Steinhaus, *Studia Math.*, 7, 1–15 (1938).

## TRANSFORMATION THEORY OF PHYSICAL CURVES

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1. The importance of projective transformations<sup>1</sup> in dynamics was brought out by Appell in 1889. If  $(\phi, \psi)$  are the rectangular components of any positional field of force in the plane, the corresponding equations of motion of a particle of unit mass are

$$\frac{d^2x}{dt^2} = \phi(x, y), \quad \frac{d^2y}{dt^2} = \psi(x, y). \quad (1)$$

If an arbitrary point transformation, unaccompanied by any change in the time, is applied, the new differential equations will usually involve not only  $x$  and  $y$ , but also the velocity components  $dx/dt$ ,  $dy/dt$ . In fact, the only exception is where the point transformation is merely affine.

Appell showed that if a general collineation

$$X = \frac{a_1x + b_1y + c_1}{a_0x + b_0y + c_0}, \quad Y = \frac{a_2x + b_2y + c_2}{a_0x + b_0y + c_0}, \quad (2)$$

is accompanied by a change of the time of the form

$$dT = \frac{dt}{k(a_0x + b_0y + c_0)^2} \quad (3)$$

the new differential equations will be of the original form

$$\frac{d^2X}{dT^2} = \Phi(X, Y), \quad \frac{d^2Y}{dT^2} = \Psi(X, Y), \quad (4)$$

and therefore define motion in some new positional field of force. The relation between the new field and the original field is explicitly as follows

$$\begin{aligned} \Phi &= k^2(a_0x + b_0y + c_0)^2[C_2(y\phi - x\psi) + B_2\phi + A_2\psi], \\ \Psi &= k^2(a_0x + b_0y + c_0)^2[C_1(y\phi - x\psi) + B_1\phi + A_1\psi], \end{aligned} \quad (5)$$

where the capital letters denote minors in the determinant  $(a_1b_2c_0)$ .

The trajectories of the original field are converted by the collineation into the trajectories of the new field. Also the directions of the forces are projectively related. However, the vector transformation induced by the collineation between the two fields of force is *not* projective, but is actually a cubic transformation.

2. Appell proved the following converse theorem. The only transformations of the form

$$X = X(x, y), \quad Y = Y(x, y), \quad dT = F(x, y)dt, \quad (6)$$

which convert every set of differential equations of the form (1) into one of the same form are those defined by (2) and (3).

3. Upon eliminating the time  $t$  from the differential equations (1), the differential equation of third order defining the  $\infty^3$  trajectories of a positional field of force is

$$(\psi - y'\phi)y''' = [\psi_x + (\psi_y - \phi_x)y' - \phi_y y'^2]y'' - 3\phi y''^2. \quad (7)$$

Kasner proved that *the only point transformations (or contact transformations) which convert every trajectory system of a positional field of force defined by a third order differential equation of the form (7) into a trajectory system of the same form are the collineations.*<sup>2</sup>

4. We shall consider the transformation theory of other noteworthy systems of curves connected with a positional field of force, namely, brachistochrones, catenaries and velocity curves. In order to do this in a simple manner, it is found convenient to discuss the transformation theory of certain systems  $S_k$  which include the four systems of physical interest as special cases.<sup>3</sup>

A system  $S_k$  in a given positional field of force consists of curves along which a constrained motion is possible so that the pressure  $P$  is proportional to the normal component  $N$  of the force vector. Thus  $P = kN$ . The differential equation of third order defining a system  $S_k$  of  $\infty^3$  curves is

$$\begin{aligned} (\psi - y'\phi)y''' &= [\psi_x + (\psi_y - \phi_x)y' - \phi_y y'^2]y'' \\ &\quad - \left[ 3\phi + \frac{(n-2)(\phi + y'\psi)}{1 + y'^2} \right] y''^2, \end{aligned} \quad (8)$$



where

$$n = \frac{2}{k+1}. \quad (9)$$

The four cases of physical interest are

$k = 0$  or  $n = 2$  gives  $S_0$ , the system of *trajectories*.

$k = -2$  or  $n = -2$  gives  $S_{-2}$ , the system of *brachistochrones*.

$k = 1$  or  $n = 1$  gives  $S_1$ , the system of *catenaries*.

$k = \infty$  or  $n = 0$  gives  $S_\infty$ , the system of *velocity curves*.

5. We shall establish the following result.

*Except for the system  $S_0$  of trajectories, the only point transformations (or contact transformations) which convert every system  $S_k$  of curves into a system  $S_k$  are the similitudes.*

In particular, the similitudes are the only contact transformations which carry every system of brachistochrones, or catenaries, or velocity curves into a system of brachistochrones, or catenaries, or velocity systems, respectively.

6. In order to prove this result, we observe in the first place that any system  $S_k$  possesses the Property I (that is, the focal locus of the osculating parabolas of the trajectories passing through a given lineal element  $E$  is a circle passing through the point of  $E$ ). Any system of curves with the Property I is said to be of the type (G). This (G) type is defined by the differential equation of third order

$$y''' = G(x, y, y')y'' + H(x, y, y')y''^2. \quad (10)$$

Kasner proved that *the only contact transformations which send every system of  $\infty^2$  curves of the type (G) into a system of the type (G) are the collineations and correlations.*

7. We shall verify that the collineation (2) actually leaves the type (G) unchanged.<sup>4</sup>

The first three extensions of the collineation (2) are

$$\begin{aligned} Y' &= \frac{dY}{dX} = \frac{C_1(y - xy') + B_1 + A_1y'}{C_2(y - xy') + B_2 + A_2y'} \\ Y'' &= \frac{d^2Y}{dX^2} = (a_1b_2c_0)\Omega^2y'', \quad \Omega = \frac{a_0x + b_0y + c_0}{c_2(y - xy') + B_2 + A_2y'} \\ Y''' &= \frac{d^3Y}{dX^3} = (a_1b_2c_0)(a_0x + b_0y + c_0)\Omega^4y''' + \\ &\quad 3(a_1b_2c_0)(xC_2 - A_2)\Omega^3y''^2 + 3(a_1b_2c_0)(a_0 + b_0y')\Omega^4y''. \end{aligned} \quad (11)$$

Let the (G) type in the  $(X, Y)$ -plane be

$$Y''' = \bar{G}(X, Y, Y')Y'' + \bar{H}(X, Y, Y')Y''^2 \quad (12)$$

By (11), it is found that under a collineation this corresponds to a (G) type (10) in the  $(x, y)$ -plane. The correspondence between the defining functions is

$$G = \frac{1}{a_0x + b_0y + c_0} \left[ \frac{\bar{G}}{\Omega} - 3(a_0 + b_0y') \right],$$

$$H = \frac{\Omega}{a_0x + b_0y + c_0} [(a_1b_2c_0)\Omega\bar{H} - 3(xC_2 - A_2)]. \quad (13)$$

8. The calculation for correlations is simplified by observing that every correlation may be reduced by means of collineations to Legendre's transformation

$$X = -y', \quad Y = xy' - y, \quad Y' = -x. \quad (14)$$

This is polarity with respect to the conic  $x^2 + 2y = 0$ . Extending this, we find

$$Y'' = \frac{1}{y''}, \quad Y''' = \frac{y'''}{y''^3}. \quad (15)$$

This converts the (G) type (10) into the (G) type (12) where the new coefficient functions are related to the old as follows

$$G = \bar{H}(-y, xy' - y, x), \quad H = \bar{G}(-y, xy' - y, -x). \quad (16)$$

Thus we have verified that the collineations and correlations leave the (G) type unchanged. Therefore the only possible contact transformations that send every system  $S_k$  of  $\infty^3$  curves into a system  $S_k$  are the collineations and correlations.

9. Next we observe that any system  $S_k$  possesses the Property II, which may be stated in the following manner. At any point  $O$ , the tangent of the angle which the focal circle makes with the given element is to the tangent of the angle which the given element makes with a certain direction fixed at  $O$  of slope  $\omega(x, y)$  (the direction of the acting force), as 3 is to  $n + 1$ , that is, as  $3k + 3$  is to  $k + 3$ . This means analytically that  $H$  has the form

$$H = \frac{3}{y' - \omega} + \frac{(n - 2)(1 + y'\omega)}{(1 + y'^2)(y' - \omega)}. \quad (17)$$

*It is evident that the correlations do not preserve systems with Properties I and II. For  $H$  and  $G$  are interchanged under the Legendre transformation and hence the special function  $H$  becomes an arbitrary function  $G$ .*

*Finally the only collineations which preserve the systems with Properties*

$I$  and  $II$  where  $k \neq 0$  or  $n \neq 2$  are the similitudes. For by (17),  $Y' = \pm i$  renders  $\bar{H}$  infinite. Then by the last of equations (13), the pre-images of the minimal lines  $Y' = \pm i$  must make  $H$  infinite. But we know by (17) that  $y' = \pm i$  are infinite roots of  $H$ . Thus the minimal lines  $y' = \pm i$  must be converted into the minimal lines  $Y' = \pm i$  by the collineation (2) (unless the field of force has constant slope  $\pm i$  which possibility is excluded from consideration). Therefore any such collineation is necessarily a similitude.

As it is evident that similitudes do preserve the systems  $S_k$ , it follows that the proof of the Theorem of Section 5 is complete.

<sup>1</sup> Appell, *Amer. Jour. Math.*, 1889. The importance of the conformal group in dynamics (conservative fields of force) has been emphasized by Larmor, Goursat and Darboux.

<sup>2</sup> Kasner, *Differential-Geometric Aspects of Dynamics*, Princeton Colloquium Lectures, American Mathematical Society Publications 1913, 1934.

<sup>3</sup> Kasner, "Physical Curves," these PROCEEDINGS, 33, 346-351 (1947).

<sup>4</sup> Kasner, "The Trajectories of Dynamics," *Trans. Amer. Math. Soc.*, 7, 401-424 (1906).

## THE ENERGY SOURCE FOR BIOLUMINESCENCE IN AN ISOLATED SYSTEM

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When luminous organisms are extracted with water or saline solutions one usually obtains in the crude preparation a temporary emission of light which disappears rapidly. In all but five of some twenty-one groups of luminous forms the light-emitting system is destroyed by the extraction procedures. Some of the difficulties are exemplified by the system which has been extracted from the crustacean, *Cypridina hilgendorffii*. Here, Harvey<sup>1</sup> demonstrated that the substrate for the reaction, luciferin, was highly unstable in the presence of air. The oxidation which occurred, however, could be reversed by simple reductants provided the compound did not remain in the oxidized state for too long a period. In the presence of the enzyme, luciferase, however, an oxidation occurred, accompanied by light production, which was not reversible by simple reductants.<sup>2</sup> The degradation of a ketohydroxy side chain on the luciferin molecule has been presented as a possible explanation of this irreversible reaction.<sup>3</sup> Since purified *Cypridina* luciferin was shown to contain labile phosphate, it has been postulated that the energy derived from the breakdown of the side chain is conserved as phosphate bond energy.<sup>4</sup> This suggestion accounts

satisfactorily not only for the irreversible reaction observed *in vitro* but also for the energy requirements of luminescence. The hypothesis was supported by the observation that phosphate was released during the luminescent reaction.

Recently results have been obtained with Lampyrid beetles, popularly known as "fireflies," which support the early suggestion that labile phosphate is concerned in the luminescent reaction. When live fireflies (*Photinus pyralis*) are ground with sand and water in a mortar one obtains momentarily a preparation which is highly luminous; however, the light disappears rapidly with continued grinding. Presumably the non-luminous extract contains the enzyme luciferase, and the luciferin which has been irreversibly decomposed. However, if one adds a small amount of adenosine triphosphate (ATP)<sup>6</sup> to this crude extract a brilliant flash of light appears immediately and lasts for a considerable time depending on the ATP concentration. The results summarized in figure 1 showing the

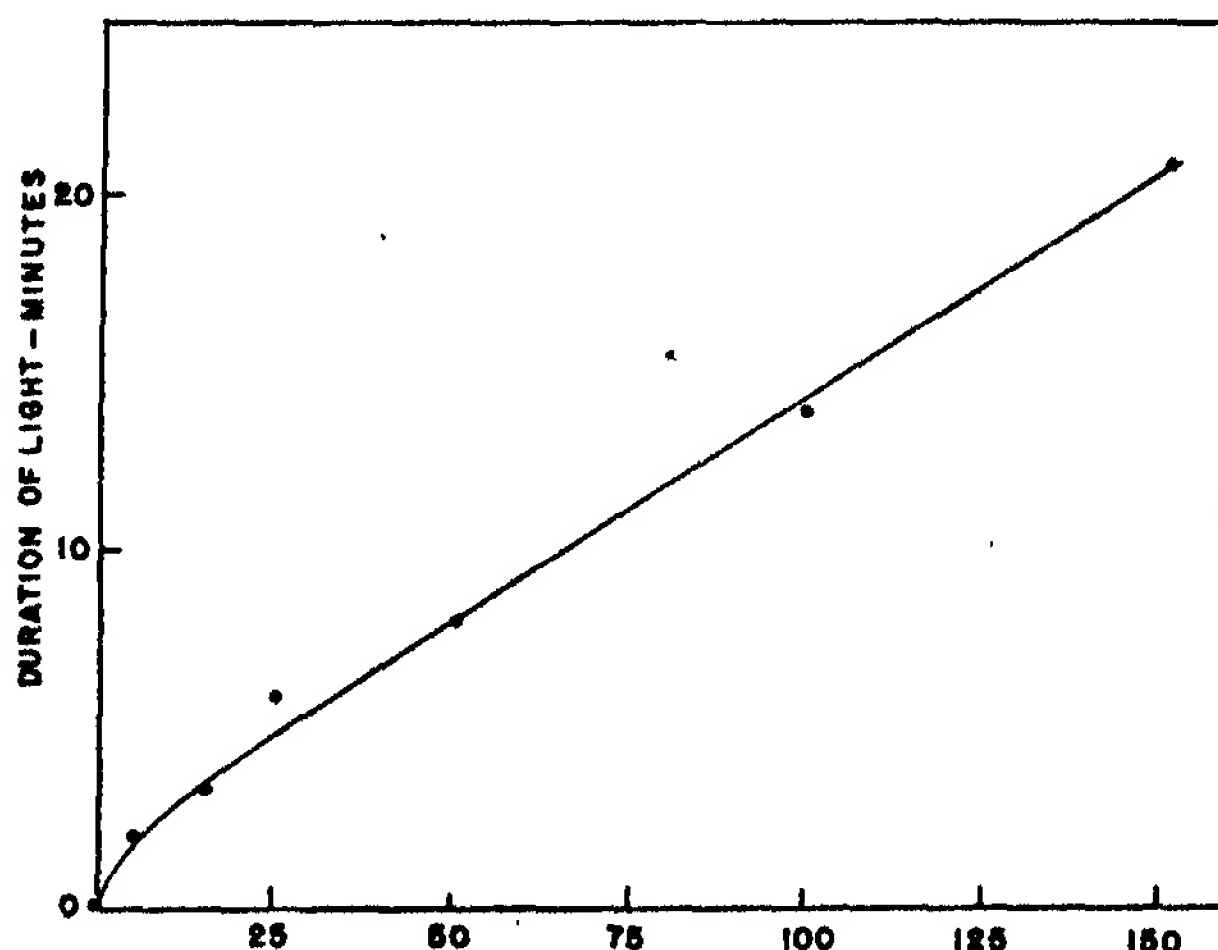


FIGURE 1

The relationship between light emission and adenosine triphosphate

relationship between ATP concentration and duration of light were obtained with the following preparations. The fireflies were placed for 30 minutes in absolute ethyl alcohol containing dry ice and then dried under vacuum in the frozen state. After drying, the lanterns were removed from 50 fireflies and ground with sand and 15 ml. of cold water for 10 minutes. The preparation was centrifuged for 20 minutes (R.C.F. = 2000) and the supernatant, which presumably contained luciferin, was saved. To

obtain the enzyme 50 live fireflies were ground with sand and 50 ml. of water and finally centrifuged as above and the supernatant saved. When the two extracts were combined no light appeared. However, with the addition of ATP the entire preparation gave what appeared to be a very homogeneous glow. In the above experiments 0.1 ml. of the substrate was mixed with 0.1 ml. of enzyme solution. Water or ATP was added to give a final volume of 0.7 ml.

At the present time it is not possible to measure the total amount of light emitted by a given concentration of ATP. Therefore, one cannot make a quantitative comparison between light and ATP. With low concentrations of ATP a very bright light is first observed which then decays slowly and finally disappears. With higher concentrations of ATP a bright light is maintained fairly constant for a while and then decays slowly. Consequently, with low concentrations of ATP which maintain the light for only a few minutes a large percentage of the observation time is concerned with the decay part of the curve in contrast to higher concentrations in which the decay portions of the curve represent only a small percentage of the total time.

In the above preparation it was observed that the luciferase preparation itself could be made to luminesce if ATP were added to it. However, the alcohol-treated preparation did stimulate the luciferase preparation and a much brighter light was obtained. Like *Cypridina*, however, it is possible to dialyze the crude extract and obtain a diffusible, temperature-stable substrate and a non-diffusible temperature-labile substance, which when mixed in the presence of ATP will emit light. The dialyzed luciferase preparation did not emit light when ATP was added. The difficulty of dialyzing the enzyme free of the substrate, however, indicates that the combination is a fairly strong one, resembling certain prosthetic groups and apoenzyme combinations. It is possible to concentrate the dialyzable substance by evaporation, and attempts are being made at the present time to identify the compound or compounds concerned.

Since the above observations were made it has been found that the light emitting system can be concentrated from a fresh aqueous extract of the fireflies simply by drying the extract under vacuum. Likewise, whole flies can be dried under vacuum and an extract made of the dried lanterns. However, if the whole fireflies are kept in the dried state for several days the extracted system cannot be made to luminesce with the addition of ATP even though a bright light is obtained during the extraction procedure. This is in contrast to the dried extract made from fresh material which always emits light with the addition of ATP, even after remaining in the dried state for over a month. It has also been observed that oxygen is necessary for light emission in the firefly extract, for when the various components are mixed under anaerobic conditions no light is

obtained. With the admission of air, however, the usual bright glow appears immediately.

It is not possible at the present time to say whether ATP is concerned directly or indirectly with light production. With the crude preparations it is conceivable that ATP is a necessary component for some system which in turn "re-activates" the luciferin. However, the earlier observations that acid-labile phosphate is present in the purified *Cypridina* luciferin preparations and that phosphate is released concomitantly with light emission lead one to suspect that the "energy rich" phosphate groups are directly concerned in exciting the luciferin molecule. On the basis of the previous hypothesis, then, it is suggested that the light-emitting reaction is reversed by the transfer of phosphate from ATP to the luciferin molecule, the energy requirements for light emission being satisfied primarily by phosphate bond energy. This suggests that luciferin may act in both phosphate and electron transporting systems.<sup>6</sup>

\* I am indebted to Miss Helma Miller and Messrs. R. E. Anderson, Abdel-Hamid Farghaly, G. de la Haba and E. Daniel for their invaluable assistance in collecting the fireflies.

<sup>1</sup> The numerous studies on bioluminescence are discussed by E. Newton Harvey, *Living Light*, Princeton University Press, Princeton, N. J., 1940.

<sup>2</sup> Anderson, R. S., *J. Gen. Physiol.*, **19**, 301-305 (1935).

<sup>3</sup> Chakravorty, P. N., and Ballentine, R., *J. Am. Chem. Soc.*, **63**, 2030 (1941).

<sup>4</sup> McElroy, W. D., and Ballentine, Robert, *Proc. Nat. Acad. Science*, **30**, 377-382 (1944).

<sup>5</sup> The adenosine triphosphate was prepared from rabbit muscle as the barium salt according to the method of Needham, using the modifications given in Umbreit, W. W., Burris, R. H., and Stauffer, J. F., *Manometric Techniques*, Burgess Publishing Co., Minneapolis, 1945. According to the 7 minute phosphate and purine analysis the preparation was 85% pure.

<sup>6</sup> I wish to thank Drs. E. N. Harvey, A. M. Chase, R. S. Anderson and Robert Ballentine for suggestions made in the preparation of the manuscript and Dr. John B. Buck for identifying the species of fireflies.





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## *STUDIES ON THE BIOCHEMISTRY OF TETRAHYMENA. X. QUANTITATIVE RESPONSE TO ESSENTIAL AMINO ACIDS\**

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*Introduction.*—Previous studies<sup>1-7</sup> have shown that the ciliated protozoan, *Tetrahymena*, has nutritional requirements very similar to animals in general. This similarity is especially striking in regard to amino acid requirements where the ten essential amino acids of the dog, man, etc., have been shown to be essential also for *Tetrahymena*. Inasmuch as this protozoan possesses characteristics which make its culture in the absence of all other organisms relatively simple, it has been possible to investigate rather precisely the effects of various substances on its metabolism. Biochemical studies of this nature on other animals have been complicated and even vitiated by contaminating microorganisms of unknown synthetic abilities.

This report deals with the growth responses of *Tetrahymena* to varying concentrations of the essential amino acids and to serine, and includes the results of tests involving availability of natural and unnatural isomers.

*Experimental.*—The organism used in this investigation was *Tetrahymena geleii* W grown in pure (bacteria-free) culture. The general techniques have been previously reported.<sup>1, 4, 8</sup> Dose-response curves were constructed for each of the essential amino acids, and for the stimulatory amino acid, serine, by adding graded amounts to a base medium containing the other ten in the proportions found in gelatin (table 1, medium II) together with growth factors, dextrose and salts. The minimum concentrations which permitted optimum growth under these conditions were combined, but the nitrogen level was then inadequate so these amounts were increased fivefold (medium III, table 1). Dose-responses were then studied using these new proportions. This medium was also used in studies on the availability of unnatural isomers.

The factor II preparations, which are necessary for the growth of this organism<sup>9, 10</sup> were of two types. The one prepared from cerophyl, as

TABLE 1

AMINO ACID	MICROGRAMS PER ML.	
	MEDIUM II (BASED ON GELATIN)	MEDIUM III (BASED ON GROWTH OPTIMA)
L-arginine HCl	820	125
L-histidine HCl	100	125
DL-isoleucine	350	125
L-leucine	350	250
L-lysine	600	250
DL-methionine	340	500
DL-phenylalanine	140	350
DL-threonine	200	125
L-tryptophane	100*	50
DL-valine	200*	125
DL-serine	40	250

\* Amount arbitrarily added.

Both media contained the following substances (micrograms per ml.):

Dextrose.....	1000	Thiamine HCl.....	1.00
MgSO <sub>4</sub> ·7H <sub>2</sub> O.....	100	Nicotinamide.....	0.10
K <sub>2</sub> HPO <sub>4</sub> .....	100	Pyridoxine HCl.....	0.10
CaCl <sub>2</sub> ·2H <sub>2</sub> O.....	50	Riboflavin.....	0.10
FeCl <sub>3</sub> ·6H <sub>2</sub> O.....	1.25	Pteroylglutamic acid.....	0.01
MnCl <sub>2</sub> ·4H <sub>2</sub> O.....	0.05	Choline Cl.....	1.00
ZnCl <sub>2</sub> .....	0.05	Yeast nucleic acid (hydrolyzed)...	100.00
Biotin (free acid).....	0.0005	Factor II (see text)	
Ca pantothenate.....	0.10		

previously described,<sup>2</sup> was assayed quantitatively with *Leuconostoc mesenteroides* P-60, according to the method of Shankman, *et al.*,<sup>11</sup> and the following amounts of the ten essential amino acids were found (expressed in  $\gamma$  per ml. of final *Tetrahymena* medium): arginine, 0.9; histidine, 0.6; isoleucine, 0.0; leucine, 0.8; lysine, 0.2; methionine, 0.0; phenylalanine, 7.0; threonine, 0.0; tryptophane, 0.38; valine, 0.0. This preparation was satisfactory for all of the amino acids studied except phenylalanine. A factor II preparation which assayed 0.4  $\gamma$  of phenylalanine per ml. of medium was used in the studies involving this amino acid. The starting material in this case was Liver Fraction L† (15 g.). This was dissolved in 750 ml. of water and extracted with butanol for 96 hours in a liquid-liquid extraction apparatus.<sup>8</sup> After removal of the butanol by distillation *in vacuo* the volume was adjusted to 300 ml. and treated with Norit at pH 5.0. The final preparation was used in a concentration of 1:10.

All determinations were made on third serial transplants after 72 hours' incubation at 25°C. when serine was present, or 144 hours' incubation when serine was absent.

**Results.—Serine.**—As has been shown previously<sup>1</sup> serine is a general growth stimulator for *Tetrahymena* and this stimulation appears to be due to its ability to release inhibitions caused by the essential amino acids. It

will be shown later in this paper that serine plays a multiple rôle in growth, involving, together with the release of specific inhibitions, efficient use of certain of the amino acids. These relationships will be discussed with the specific amino acids concerned.

When serine is omitted from medium III no growth results but as little as 10  $\gamma$  per ml. (table 2) of either natural or racemic serine provides maximum stimulation (range tested 0–250  $\gamma$  per ml.). Both isomers are active. On the other hand, omission of serine together with a lowering of the concentration of any one of the essential amino acids (with the single exception of threonine) permits growth to occur, although at a greatly reduced rate and submaximal yield (table 3). This means that serine must be present to release the inhibition exhibited by each of the essential amino acids in the concentrations used. But the organism can partially replace the exogenous serine effect, provided that the concentration of at least one of the inhibitory amino acids is lowered.

TABLE 2

MINIMUM CONCENTRATION REQUIRED FOR MAXIMUM RESPONSE WHEN TESTED WITH ALL OF THE OTHER AMINO ACIDS IN THE CONCENTRATIONS OF MEDIUM III. FIGURES REPRESENT MICROGRAMS PER ML.

AMINO ACID	SERINE PRESENT		SERINE ABSENT	
	L	DL	L	DL
Arginine	20	...	0	...
Histidine	7.5	...	7.5	...
Isoleucine	17.5	25	200	No growth
Leucine	25	140	25	140
Lysine	15	15	15	15
Methionine	20	30	20	30
Phenylalanine	10	40	10	40
Threonine	10	15	225	225
Tryptophane	10	12	10	12
Valine	2.5	7.5	0	0
Serine	10	10		

*Arginine and Valine.*—It was shown early<sup>2</sup> that *Tetrahymena* could, under certain conditions, synthesize slowly both arginine and valine. Both of these amino acids are stimulatory but only in the presence of serine, which is to say that in the absence of serine growth is not improved when these amino acids are added to the medium at any level. The level giving maximum response for L-arginine is 20  $\gamma$  per ml. (table 2) when serine is present, but no amount of added arginine stimulates growth in its absence (range tested 0–125  $\gamma$ ). Both natural and racemic valine were tested and, as in the case of arginine, no amount of either proved stimulatory in the absence of serine while maximum response was obtained, with serine present, with 2.5  $\gamma$  per ml. of L-valine and 7.5  $\gamma$  per ml. (table 2) of DL-valine (range tested L—0–35  $\gamma$  per ml.; DL—0–250  $\gamma$ ). These quantita-

tive relationships indicate that only the natural form of valine is active for *Tetrahymena*, and that the D-isomer is somewhat inhibitory.

Tests were conducted to determine the cause of the failure of valine synthesis when tested with medium II. Additions of quantities of each amino acid to medium III so as to adjust its level to that of medium II showed that the high levels of arginine and lysine in the older mixture were responsible for blocking the synthesis of valine. Earlier observations<sup>2</sup> have indicated that this block can be partially released by adding glycine, serine and cystine together.

TABLE 3

SUMMARY OF THE QUANTITATIVE RELATIONS BETWEEN SERINE AND THE ESSENTIAL AMINO ACIDS

Column I represents the concentrations in Medium III. Changes in concentrations are indicated in bold faced type

AMINO ACID	MICROGRAMS PER ML.											
	I	II	III	IV	V	VI	VII	VIII	IX	X	XI	XII
DL-serine	250	0	0	0	0	0	0	0	0	0	0	0
L-arginine	125	125	0	125	125	125	125	125	125	125	125	125
L-histidine	125	125	125	7.5	125	125	125	125	125	125	125	125
DL-isoleucine	125	125	125	125	*	125	125	125	125	125	125	125
L-leucine	250	250	250	250	250	80	250	250	250	250	250	250
L-lysine	250	250	250	250	250	250	15	250	250	250	250	250
DL-methionine	500	500	500	500	500	500	500	30	500	500	500	500
DL-phenylalanine	350	350	350	350	350	350	350	350	50	350	350	350
DL-threonine	125	125	125	125	125	125	125	125	125	225	125	125
L-tryptophane	50	50	50	50	50	50	50	50	50	50	10	50
DL-valine	250	250	250	250	250	250	250	250	250	250	250	0
Growth†	+	0	+	+	0	+	+	+	+	+	+	+

\* Neither lowering nor raising the concentration of DL-isoleucine permitted growth in the absence of serine, provided the concentrations of the other amino acids remained as in Column I. The substitution of 275 micrograms of L-isoleucine, however, permitted growth in the absence of serine.

† The plus sign (+) represents transplantable growth, the zero (0) represents no growth. The plus signs are not necessarily equal, as growth is slow and never maximum in the absence of serine, except where threonine levels are high (Column X).

*Histidine, Lysine, Methionine, Tryptophane.*—This group of essential amino acids will be considered together because they all behave similarly in relation to serine, although they differ in certain other details. In the absence of serine, growth occurred when the level of any one of these amino acids was lowered sufficiently (table 3) but the growth rate was low, as was the maximum yield. The maximum response levels were the same as those found in the presence of serine (table 2). Only natural histidine was available for testing and maximum response was obtained with the addition of 7.5  $\gamma$  per ml. of medium (range tested 0–125  $\gamma$ ). Both isomers

of lysine are active. The maximum response was obtained at 15  $\gamma$  per ml. of either L- or DL-lysine (range tested 0–300  $\gamma$ ). Both isomers of methionine appeared active, as the amounts required for maximum response were 30  $\gamma$  and 20  $\gamma$  per ml. of the DL- and L-form, respectively (range tested L—0–90  $\gamma$ ; DL—0–500  $\gamma$ ). This seems to indicate some inefficiency in the ability to metabolize the D-isomer. This condition is apparent also in the case of tryptophane where maximum response was obtained with 10  $\gamma$  of L-tryptophane and with 12  $\gamma$  of the racemic mixture (range tested L—0–20  $\gamma$ ; DL—0–50  $\gamma$ ).

*Phenylalanine, Leucine.*—When the concentration of phenylalanine is reduced in the absence of serine, low but transplantable growth results (table 3). The maximum response levels, either with or without serine, are identical but wide differences exist between the activities of the natural and racemic forms. Maximum response was obtained with 10  $\gamma$  (table 2) of L-phenylalanine (range tested 0–140  $\gamma$ ) while 40  $\gamma$  of the racemic mixture are required (range tested 0–350  $\gamma$ ). The activity of leucine is similar to phenylalanine. The maximum response levels either with or without serine were identical but, again, large amounts of the racemic mixture were required (25  $\gamma$  of L-leucine; 140  $\gamma$  of DL-leucine, range tested 0–700  $\gamma$ ). These large differences between the natural and racemic mixtures indicate competitive inhibition by the D-isomers and is found also in the case of valine, mentioned above. The natural form of leucine became inhibitory at levels above 125  $\gamma$  per ml. when serine was absent but very high levels (700  $\gamma$  per ml.) were tolerated when serine was present.

*Isoleucine.*—In the presence of serine both isomers of isoleucine appear to be metabolized, although the D-isomer somewhat inefficiently. Maximum response was obtained with 17.5  $\gamma$  per ml. of the natural form and 25  $\gamma$  per ml. of the racemic mixture (range tested 0–400  $\gamma$ ). In the absence of serine no growth was possible with any concentration of DL-isoleucine (range tested 0–400  $\gamma$ ). The D-isomer in this case appears to be completely inhibitory. Utilization of the L-isomer is faulty without serine as the growth was always slow, the yields low and the amounts required high (200  $\gamma$  per ml.). These results indicate that serine, in addition to counteracting inhibition, makes isoleucine available for *Tetrahymena*. Unlike leucine, the natural form of isoleucine showed no inhibition without serine, in the range tested. Growth at levels of 400  $\gamma$  per ml. was as good as at 200  $\gamma$  per ml.

*Threonine.*—As with methionine, isoleucine and tryptophane, the unnatural isomer of threonine appears to be utilized (when serine is present) but not as efficiently as the natural form. Maximum response was obtained with 10  $\gamma$  per ml. and 15  $\gamma$  per ml. of L-threonine and DL-threonine (table 2), respectively (range tested 0–400  $\gamma$  per ml.). In the absence of serine no growth occurred at these levels. Unlike the other amino acids,



high levels of threonine are not inhibitory but rather substitute for serine in the release of inhibition (table 3). The maximum yield with 225  $\gamma$  per ml. of either natural or racemic threonine was as high as when serine was present, although, as in every case above noted, the growth rate was reduced. Release of inhibition apparently is not influenced by the stereo-configuration of threonine any more than it is by serine.

*Discussion.*—The results of this study raise more questions than they answer. The complexity of the interactions between amino acids is perhaps not surprising when their general lability in metabolism is taken into consideration. Nevertheless, a number of the facts brought out should be considered if for no other reason than to attempt explanations which may be subject to experimental test.

Release of inhibition by serine requires some elaboration. It was stated that, under the conditions of these experiments, the reduction of any one of the amino acids except threonine reduced the total inhibition to a point where growth could occur without serine. An apparent exception to this statement was found with DL-isoleucine. No concentration of DL-isoleucine allowed growth to occur in the absence of serine, with low threonine, and the other amino acids high. It appears that non-inhibitory concentrations of isoleucine are insufficient for metabolic needs. Inhibitory concentrations of DL-isoleucine were always present (concentration of medium III, 125  $\gamma$  per ml.) when the other amino acids were being tested, but this concentration was not enough to stop growth when their levels were reduced. It was obvious, therefore, that growth should occur at some level using DL-isoleucine if some other amino acid level were lowered. This was found to be the case. For example, if L-leucine was reduced from 250  $\gamma$  to 80  $\gamma$  per ml. and a dose response curve for DL-isoleucine constructed, it was found that high maximum yields were obtained with 25  $\gamma$  per ml. of the latter. The growth rate was slow, as was expected in the absence of serine. High concentrations (350  $\gamma$  per ml. and above) of the racemic isoleucine were completely inhibitory. The same general results were obtained when phenylalanine was reduced (from the 350  $\gamma$  per ml. of medium III to 50  $\gamma$  per ml.) or the threonine level raised (from 125  $\gamma$  per ml. to 300  $\gamma$  per ml.). Lowering of phenylalanine inhibition or raising the threonine concentration made even 400  $\gamma$  per ml. of DL-isoleucine not completely inhibitory.

When one examines the summary given in table 3, one can see that growth resulted in the absence of serine, only when individual amino acid concentrations were lowered, with the single exception of threonine (Column X). That threonine can substitute for serine is perhaps understandable on the basis of their structural similarities.

In view of the fact that earlier work<sup>2</sup> had shown that glycine sometimes functions to antagonize inhibitions and that acetate in low concentrations

is stimulatory,<sup>†</sup> and also that serine is readily converted to glycine, medium III minus serine was tested with graded amounts of glycine (range tested 0–200  $\gamma$  per ml.). Again no growth occurred when only the ten essential amino acids were present, but growth did occur in the presence of glycine. Very low growth resulted with the addition of as little as 2.5  $\gamma$  per ml. and steady increases occurred up to 50  $\gamma$  per ml. Maximum yield at 50  $\gamma$  per ml. was as high as when 10  $\gamma$  per ml. of serine was added, but the growth rate was low, in contrast to serine. These quantitative relationships indicate that serine does not function by conversion to acetate via glycine.

In an attempt to improve growth in the absence of serine, and yet use the same isomeric forms of the amino acids as in medium III, a medium was constructed with the following concentrations (in  $\gamma$  per ml.): L-arginine, 40; L-histidine, 50; DL-isoleucine, 50; L-leucine, 100; L-lysine, 50; DL-methionine, 60; DL-phenylalanine, 50; DL-threonine, 300; L-tryptophane, 20; DL-valine, 7.5. The amino acids totaled 727.5  $\gamma$  per ml. as compared to 2275  $\gamma$  per ml. for medium III, and the amino nitrogen totaled 100.8  $\gamma$  per ml. as compared to 308.6  $\gamma$  per ml. for medium III. With this medium growth yields were nearly as great as when serine was added, but still the rate of growth was somewhat slow. Inhibition, as far as yield was concerned, was largely removed by the combined effect of high threonine and low concentrations of all of the other amino acids. The somewhat lower yield was possibly due to nitrogen limitations and the low growth rate due to faulty metabolism of isoleucine in the absence of serine.

Turning now to a consideration of the results on the utilization of the isomers, the most interesting and puzzling seem to be those with leucine, phenylalanine and valine. Even in the presence of serine *more* than double the amounts of the racemic mixtures than of the natural forms are required for maximum response. It appears that the D-isomers are inhibitory and unavailable but that this inhibition can be overcome, even though the 50–50 ratio of L to D is maintained, if larger amounts are added. One possible explanation is on the basis of competitive enzyme inhibition. At low levels of the racemic mixture 50% of the enzymes responsible for metabolism of the amino acid in question take up the available L-isomer and 50% D-isomer. As this binding of the enzyme by the D-isomer is reversible (according to the theory of enzyme competition) and the L-isomer is being used up, then at no time will the enzyme be able to mobilize enough L-isomer for adequate growth. As the concentration of both isomers is increased, however, there finally results a condition where 50% of the enzyme can at all times be saturated with the L-isomer, which, on the basis of margin of safety, is sufficient for optimum growth. If this is the true explanation for the results obtained with leucine, phenylalanine and valine, then we will have to assume enzyme action on some position of the molecule other than the asymmetric carbon, for these amino acids.

The apparent inability of this organism to metabolize efficiently the D-isomers of isoleucine, methionine, threonine and tryptophane cannot be explained at this time. In the case of methionine it may be that the less than double amounts of the racemic mixture as compared to the natural form for maximum response might point to the well-known double function of this amino acid: as an essential amino acid and as a methyl donor. As a methyl donor it may make no difference what the configuration is and therefore some of the D-isomer can be used to spare the L-isomer. Similar double functions, as yet unknown, may explain the results with the other three amino acids under discussion, i.e., niacin synthesis in relation to tryptophane.

TABLE 4

COMPARISONS OF THE RAT, MOUSE, MAN AND *Tetrahymena* REGARDING THEIR ABILITY TO UTILIZE OPTICAL ISOMERS OF THE AMINO ACIDS. THE DATA FOR THE MAMMALS ARE TAKEN FROM A SIMILAR TABLE IN THE REVIEW BY ALBANESE<sup>12</sup>

AMINO ACID	RAT		MOUSE		MAN		<i>Tetrahymena</i>	
	L	D	L	D	L	D	L	D
Arginine	+	—	..	..	+	+	+	..
Histidine	+	+	+	+	+	—	+	..
Isoleucine	+	—	+	—	..	..	+	+
Leucine	+	+	..	..	..	..	+	—
Lysine	+	—	+	—	+	+	+	+
Methionine	+	+	+	+	+	+	+	+
Phenylalanine	+	+	+	+	+	—	+	—
Threonine	+	—	+	—	+	..	+	+
Tryptophane	+	+	+	+	+	—	+	+
Valine	+	—	+	—	..	..	+	—
Serine	..	..	..	..	..	+	+	+

Inasmuch as *Tetrahymena*, unlike all other microorganisms so far studied under like controlled conditions, identifies itself with higher animals as far as amino acid requirements are concerned, it is interesting to compare the available data on other animals regarding isomer utilization. Table 4 was constructed from a similar one given by Albanese<sup>12</sup> and details can be obtained from his review. In interpreting differences between the mouse, rat, man and *Tetrahymena*, it must not be overlooked that the intestinal flora of the vertebrates may play a decided rôle in the results obtained, while in this respect the data for *Tetrahymena* are more reliable. The results on the ciliate actually reflect *its* metabolism as all other organisms are excluded.

It has been found by Rose, *et al.*,<sup>13</sup> using nitrogen balance studies, that histidine is apparently a dispensable amino acid for man. In contrast to this histidine is indispensable for *Tetrahymena*. No growth is possible when histidine is absent from the medium, but transplantable growth re-

sults when as little as 2.5  $\gamma$  per ml. are added, and 7.5  $\gamma$  per ml. are sufficient for optimum growth. With such an active substance, there is a strong possibility that the bacterial flora of the human alimentary canal can contribute enough to account for the results of Rose, *et al.* On the contrary, under certain conditions *Tetrahymena* can synthesize valine. It is of importance, in the light of this work, to test the ability of vertebrates to dispense with valine under similar conditions of low arginine and lysine.

The results reported here, while incomplete in many respects, indicate some important points, often overlooked in studies of this nature. Even essential amino acids in moderate amounts can be inhibitory under certain conditions. This may prove of importance in oral and intravenous alimentation, especially with synthetic amino acids. The rôle of serine as a growth stimulator, in its ability to release inhibitions and to enhance the utilization of isoleucine, warrants further attention in metabolism studies. That amino acid imbalance can influence growth and reproduction is again emphasized.

*Summary.*—1. Under certain defined conditions, the optimum concentrations of the essential amino acids were determined for the ciliated protozoan *Tetrahymena*.

2. Availability of the optical isomers was investigated.

3. Quantitative results indicate that both the L- and D-isomers of lysine, methionine, threonine and tryptophane are active. Both isomers of isoleucine are active in the presence of serine.

4. The unnatural isomers of leucine, phenylalanine and valine are inhibitory.

5. High levels of L-isoleucine are required in the absence of serine and the racemic mixture is completely inhibitory.

6. Serine functions as an antagonist to the inhibitions exhibited by nine of the essential amino acids.

7. In the absence of serine the growth rate was invariably low and the maximum yield usually reduced.

8. High levels of threonine can substitute for serine for release of inhibition but threonine is not a growth rate stimulator.

9. Comparisons are made between the data in the literature on vertebrates and those reported here on *Tetrahymena*.

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† Furnished through the courtesy of Dr. David Klein and the Wilson Laboratories.

‡ Unpublished results from this laboratory.

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## EFFECT OF INCREASING FOOD PROTEIN UPON THE CALCIUM CONTENT OF THE BODY\*

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In previous papers<sup>1, 2</sup> we have noted briefly the fact that acceleration of growth by relatively high protein food may result in retardation of the body's normal developmental gain in calcium content. The purpose of the present paper is to record some further experiments bearing upon this relationship.

The experimental animals have been rats of like genetic background—an inbred laboratory stock of Wistar Strain albinos. The experiments are divided into two series according to the immediate nutritional background and the basal diet of the experimental animals.

*First Series.*—The experimental rats were separated at the age of 28 days from families fed Diet 16 (also called Diet A) consisting of five-sixths ground whole wheat and one-sixth dried whole milk with table salt in the proportion of 2% of the weight of the wheat. The air-dry food mixture contained practically 14% of protein and 0.2% of calcium. Food and water were constantly available to the animals. Rats of the same sex and essentially the same size were drawn from the same litters; in each case, one of these was continued on the basal Diet 16, while the other received Diet 16 plus casein. The casein was added in such proportion as to increase the protein content of the air-dry food mixture from approximately 14% to approximately 20%. At 60 or at 90 days of age the corresponding rats



on the two diets were killed and analyzed for body calcium. The average results of the comparisons of this series are shown in table 1.

TABLE 1  
AVERAGE CALCIUM CONTENTS OF FEMALE RATS AT 60 AND AT 90 DAYS OF AGE AS AFFECTED BY INCREASE OF FOOD PROTEIN FROM 14 TO 20%. (CALCIUM CONTENT OF FOOD ABOUT 0.2%)

	FROM DIET 16 (14% PROTEIN)	FROM DIET 16 + CASEIN (20% PROTEIN)
At 60 days of age		
Amount, g.	0.673	0.649
Percentage	0.786	0.720
At 90 days of age		
Amount, g.	1.062	0.983
Percentage	0.865	0.840

It will be seen from table 1 that both when compared at 60 and at 90 days the rats which had received the extra food protein here show less body calcium, both in amount and in percentage, than the parallel animals which had received the basal Diet 16 alone.

*Second Series.*—The arrangement of the experiments was the same in the second series as in the first except for the background and basal ration which was our Diet 133 consisting of two-thirds ground whole wheat and one-third dried whole milk with enough added calcium carbonate to bring the calcium content of the air-dry food mixture to 0.64%. This diet thus contained about three times as much calcium as the basal diet of the first series; and as it contained twice the proportion of milk it was nearly twice as rich in vitamin A and riboflavin. Its protein content was approximately 16% of the air-dry food mixture. Diet 183, fed in comparison with Diet 133, was of the same composition except that enough casein was added to bring the protein content of the air-dry food mixture from approximately 16 to approximately 20%. This increase in the level of protein feeding resulted in the production of somewhat larger young with larger amounts but somewhat lower percentages of body calcium at the age of 30 days. The average findings for these 30-day-old offspring, as well as of the original experimental animals at 60 days, 90 days and 1 year of age, are summarized in table 2. Here the animals receiving the higher amounts of food protein (and, as shown in a previous paper, growing more rapidly) showed slightly larger amounts of body calcium corresponding to their larger body weights. The slightly higher percentages at 60 and 90 days were evidently made possible by the higher calcium content of the basal diet in the second series of experiments than in the first. Continuance of growth between the ages of 90 days and 1 year involved increase in amounts of body calcium while percentages were slightly decreased by the bearing and suckling of young even though at



least a month had in each case elapsed between the end of lactation and the analysis of the mother. The year-old females on the different diets had met about equal demands of pregnancy and lactation before being taken for analysis.

TABLE 2

AVERAGE CALCIUM CONTENTS FOUND AT DIFFERENT AGES IN FEMALE RATS FROM DIETS CONTAINING 16 OR 20% OF PROTEIN IN THE AIR-DRY FOOD. (CALCIUM CONTENT OF FOOD ABOUT 0.6%)

	FROM DIET 133 (16% PROTEIN)	FROM DIET 183 (20% PROTEIN)
At 30 days of age		
Amount, g.	0.375	0.484
Percentage	1.10	0.93
At 60 days of age		
Amount, g.	1.10	1.22
Percentage	1.09	1.13
At 90 days of age		
Amount, g.	1.68	1.79
Percentage	1.23	1.29
At 1 year of age		
Amount, g.	2.62	2.69
Percentage	1.25	1.25

The chief findings of both series may be briefly summarized as follows:

Rats on a relatively low-calcium basal diet containing 14% of protein grew faster when their food protein was increased to 20%, but showed both lower amounts and lower percentages of body calcium at 60 and at 90 days of age. The differences were of the order of about one-twentieth of the amount or percentage of calcium present; and were, in this series, all in the same direction. When the basal diet was of liberal calcium content, accentuation of growth by increase of food protein did not result in lower body calcium at 60 or 90 days, nor at the age of 1 year in females which had borne and suckled young. Further analyses have confirmed and extended the finding briefly noted in previous papers from this laboratory that females of 3 months to 1 year of age which had received the low-calcium basal ration (Diet 16) plus poultry meat contained less body calcium than those of like age which had received the same basal diet without the protein enrichment. Similarity of results with poultry meat and with casein indicates that this effect of the meat was attributable to its protein. The lowering of body calcium by protein enrichment of a low-calcium diet may be regarded as an effect of a protein-calcium imbalance, or of an undue acceleration of growth, or of both.

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## *THE EFFECTS OF ISOLATES ON THE FREQUENCY OF A RARE HUMAN GENE*

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An important corollary of Mendel's principle of segregation was noted by Weinberg<sup>1</sup> and by Hardy<sup>2</sup> in 1908. They showed that in a large population undergoing random mating (panmixia), the relative frequencies of a pair of adaptively neutral alleles such as  $A$  and  $a$  tend to remain constant in the equilibrium state  $q^2AA:2q(1-q)Aa:(1-q)^2aa$  in which  $q$  and  $1-q$  are the proportions of  $A$  and  $a$  in the population. This simple relationship formed the basis for the development of the population genetics of cross-fertilizing animals and plants and has been particularly fruitful in studies of human genetics. It has been found, however, that the ideal populations assumed in the Hardy equilibrium are seldom if ever found in nature. Random mating or panmixia could hardly extend over a wide-spread animal or plant species. Human populations also are broken into rather small groups known as isolates within which marriages tend to occur at random, while genes are less frequently exchanged between the isolates. The isolating factors here may be of various kinds such as geographic, social, religious and others.

It is obvious that if random mating and free exchange of genes do not occur in large populations, the gene frequencies will be affected by circumstances peculiar to small populations, and by inbreeding or mating among relatives. Inbreeding assumes an unusual importance in human populations which generally contain recessive mutant genes for deleterious characters of various kinds. When such genes are rare, homozygotes are likely to be produced only by marriage between relatives who carry the same recessive gene. If such marriages, as between first cousins, occur at random, they will have a high probability of occurrence only when the circle of possible mates is small, as in an isolate. Therefore it is to be expected that such rare recessives will not be distributed evenly in large populations, but that different rare hereditary defects will tend to appear in different small communities, some in one, others in another.

Wahlund<sup>3</sup> (1928) has worked out, in theory, the differences to be expected in the frequencies of the several genotypes as between a whole population under random mating and one which is divided into isolates; while Dahlberg<sup>4</sup> (1943) has shown how the frequency of cousin marriages may be used to estimate the average size of the isolates, and has calculated the theoretical relationship between gene frequency, frequency of cousin

marriages in the isolate and frequency of cousin marriages among parents of persons homozygous for a given gene.

An actual example of isolates was found by Sjøgren<sup>5</sup> (1931) in studying the distribution of a gene for a rare and always fatal disease, juvenile amaurotic idiocy, as it occurs in Sweden. The disease begins with failing sight and blindness in young children of 4 to 7 years, and progresses through loss of sensory, mental and physical powers to its terminus in death some 10 or 12 years later. It was possible to detect nearly all cases occurring in Sweden since 1890, when special instruction and registration of the blind became obligatory, and to work out the ancestry and geographic origins of the affected persons by using the excellent and continuous records kept in the Swedish parishes. The heterozygous ancestors responsible for the 115 primary cases studied by Sjøgren were found to group themselves into 59 families coming from 23 rather restricted localities in southern and central Sweden; while the proportion of first cousin marriages among the parents of the amaurotics proved to be 15% which is probably 20 to 30 times the rate for the population at large. Thus the Swedish population was shown to consist, in respect to the marriages responsible for juvenile amaurotic idiots, of restricted marriage circles or isolates within which marriage between relatives occurs with higher frequency than in the population at large.

In the present note it is proposed to test some of the more recent methods by applying them to Sjøgren's data. Specifically it is of interest to determine the frequency of the gene for juvenile amaurotic idiocy in the isolates and to estimate the fraction of the whole population which is contained within the isolates.

We may begin by estimating the frequency of the gene on the assumption of random mating in the whole population of Sweden.

Sjøgren found that for the years 1913-1922, the average number of juvenile amaurotics alive at one time in Sweden was 52.5. Since the average age of onset of the disease (detected by blindness) is 6.7 years and the average age at death is 18.2 years, its average duration is about 11.5 years. Thus about  $52.5/11.5$ , or 4.6 new cases appear each year. For the ten-year period concerned, about 1,200,000 children reached the age of seven, when the disease would be detected, or about 120,000 per year. The frequency of homozygotes can thus be estimated as  $4.6/120,000$  or 0.0000383, roughly 4 per 100,000 (0.004%). Assuming random mating the proportions of homozygotes and heterozygotes at equilibrium would be  $AA = 0.9876$ ;  $Aa = 0.0123$ ;  $aa = 0.00004$ . Sjøgren recognized that the heterozygote frequency of 1.2% thus calculated for this gene could not be correct, since it applied to the whole population and must therefore be "für die Populationen in den Herdgebieten (isolates) und angrenzenden Teilen zu niedrig, für die Lakunen dazwischen zu hoch. . . ."

We must therefore calculate the gene frequency within the isolates with the aid of Dahlberg's (1943) formula,<sup>4</sup>

$$k = \frac{c(1 + 15r)}{16r},$$

in which  $k$  is the frequency of first cousin marriages among parents of juvenile amaurotics,  $c$  is the frequency of cousin marriages arising at random in the isolate population and  $r$  is the frequency of the gene. In the present case we may use for  $k$  the value of 15% found by Sjøgren, and for  $c$  the value 0.45% derived from data supplied by Professor Dahlberg on cousin marriages among parents of 17,016 children in public schools in country districts of southern and central Sweden in 1947. This gives  $r = 0.0019$ , corresponding to  $r^2 = 0.00000361$  or about 4 homozygotes per million as compared with 4 per 100,000 as found by Sjøgren. The assumption of 0.45% cousin marriages thus leads to an estimate of homozygote frequency in the isolates which is  $1/10$  of the actual frequency in the whole population in 1930. This evidently is absurd. The frequency of cousin marriages in the isolate population must therefore be higher.

Sjøgren, in considering this problem, assumed a frequency of cousin marriages of 1% and when this value is substituted in equation 1, the gene frequency  $r = 0.0044$  is found, corresponding to  $r^2 = 0.000019$  or about 2 homozygotes per 100,000. This figure is also too low. In these cases, it is probable that the frequency of cousin marriages in the isolates has been underestimated, for Sjøgren's data were chiefly from idiots diagnosed in the period 1896-1930, whose parents would therefore have been married some 30-40 years earlier when the rate of cousin marriages in restricted country districts was probably much higher. (Unpublished data of Professor Dahlberg indicate that the cousin marriage rate in the Swedish nobility declined sharply in the period 1870 to 1930.) It is probable that a cousin marriage rate of 2% in the isolates for the relevant period is not an overestimate. Using  $c = 2\%$  and  $k = 15\%$  we get a gene frequency in the isolates of 0.009524 or  $r^2 = 0.00009$ , about 1 homozygote per 10,000 in the isolates.

If we assume that all cases of juvenile amaurotic idiocy are born in the isolates, where alone the gene frequency and rate of cousin marriage are high enough to produce them, then we may estimate the fraction of the total population contained in the isolates as 42.5%. (Roughly, if the frequency of homozygotes in the whole population is about 1 per 25,000, as found by Sjøgren, and about 1 per 10,000 in the isolates, as estimated from the cousin marriage fraction, then the isolates constitute about 40% of the population.) Of the Swedish population of 6,074,368 in 1930 the isolates containing this gene thus comprised 42.5% or 2,580,000 persons. With a homozygote frequency of 0.00009 we should expect to find about 230

homozygotes in such a population. The frequency can be applied, however, only to that age fraction of the population in which juvenile amaurotic idiocy occurs, namely, in children between 7 years (onset of blindness) and 18 years (age at death). In 1930 there were 1,226,000 persons in these age groups in Sweden, or about 20% of the population. Since the chance of detection is thus 20%, we take 20% of 230, or 46, as the number of homozygotes which we may expect to find alive at one time. For the ten-year period 1912-1921 Sjøgren found an average of 52 juvenile amaurotic idiots alive at one time. The discrepancy between the actual figure and that calculated on the assumption of a cousin marriage rate of 2% and a gene frequency in the isolates of 0.009524 is not so great as to discredit the latter computation.

It is possible to estimate the average size of the isolates from the frequency of cousin marriages using the relation  $n - 1 = \frac{2b(b - 1)}{c}$  (Dahlberg, 1943), where  $n$  is the size of the isolate population,  $c$  is frequency of cousin marriage and  $b$  is number of children per family. (In the stationary Swedish population  $b$  may be taken as 2.) Where  $c = 0.02$  the isolate size is about 200. The minimum frequency of the gene could hardly be less than 2 heterozygotes per isolate, in this case a gene frequency of about 0.01, which is not far from the value 0.009 as estimated above.

There is thus a fair agreement between the frequency of homozygotes predicted by the theory and the numbers actually found by Sjøgren. It is important, however, to emphasize that even this measure of agreement is reached only by assuming a rate of cousin marriage which is much higher than that which occurs today. This is to say that the calculations apply to conditions which no longer exist and illustrates one of the difficulties in testing such theories by data from human populations. A rigid test of the methods used would require not only actual data on the cousin marriage rate but estimates of the numbers of people in some of the isolates within which the gene occurs, for comparison with predicted numbers. An attempt has been made to estimate the numbers of people in the "Heterozygoten-Herden" identified by Sjøgren but the uncertainty of isolate boundaries and the unlikeness in the population distribution in different areas have made this impractical.

Nevertheless the distribution of this rare gene, with a higher frequency in its centers, and a low or zero frequency in the rest of the population illustrates a fact about human populations which is essential to recognize. The methods for dealing with gene frequencies in isolates can be used as rough approximations, and indicate the variables which must be measured in future studies of gene distribution.

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## THE NATURE OF GENE ACTION AS SHOWN BY CELL-LIMITED AND CELL-DIFFUSIBLE GENE PRODUCTS

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The gene markers previously used to identify chromosome aberrations in the endosperm of the maize seed produce cell areas with well-defined boundaries when dominant genes are removed from the cell allowing their recessive alleles to operate. These tissue alterations are brought about during development by any process that removes chromosomes in whole or in part from the cell. The change occurs in the nucleus but the effect is visible in the cytoplasm. Isolated single cells show these changes. Obviously something passes through the nuclear membrane, either from the nucleus to the cytoplasm or in the reverse direction, but does not go beyond the cell membrane since no cell division has occurred in the single cell alterations to liberate nuclear products into the cytoplasm or the reverse.

Where the effects of the gene products are confined within the cell such genes may be considered as cell-limited. Genes of this type in maize aleurone are *C*, *R*, *Pr* and *I*. For illustration of this type of gene action see figure 1 and Jones<sup>1, 2</sup> and Clark and Copeland.<sup>3</sup>

In marked contrast to these cell-limited genes are the cell-diffusible genes where the gene products pass through the cell wall and affect adjoining cells over a considerable area. The *A*<sub>1</sub>, *A*<sub>2</sub> and *Y* color genes in maize are of this type. The *A* series of anthocyanin genes are necessary to produce color in all parts of the plant. In the recessive condition the aleurone is colorless, the cob and pericarp are brown and the other parts of the plant are green or brown, depending upon other genes present. The dominant allele produces anthocyanin in the leaves, silks, glumes, anthers, aleurone and scutellum when the complementary genes are present. When *A* is removed from the aleurone by the loss of the locus containing this gene that part of the seed is colorless. However, there is a gradual diminution in color from the pigmented to the unpigmented area extending over an area of several cells so that the border is not distinct as it is in other color changes involving *C*, *R*, *Pr* and *I*. Evidently something diffuses through



the cell walls from the pigmented cells into the unpigmented cells for a considerable distance. The colored cells bordering the uncolored area are also darker over a distance of about ten cells adjoining the uncolored area.

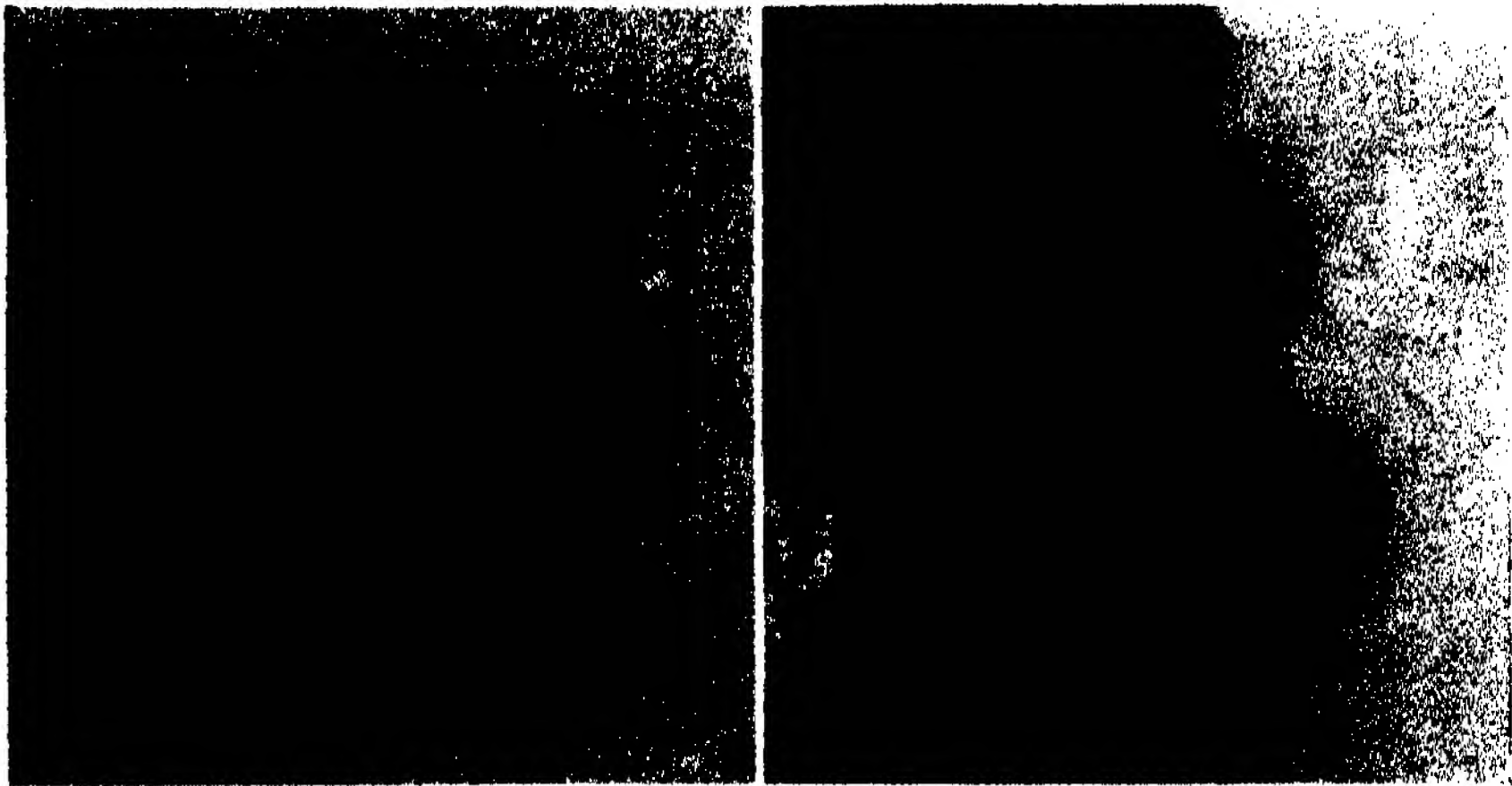


FIGURE 1

FIGURE 2

Figure 1. A change from colorless to colored aleurone cells in a maize seed resulting from the removal of a dominant color inhibitor, a cell-limited gene.

Figure 2. A change from colored to colorless aleurone cells in a maize seed resulting from the removal of the dominant color producer,  $A_1$ , a cell-diffusible gene. Note the gradation in color from dark to light areas and the darker border areas between the colored and colorless areas.

The colorless cells produce something that is not used but diffuses into the cells containing the dominant allele and forms a band of darker colored cells. See figure 2. This is an interaction between different alleles at the same locus producing an effect that is greater than that produced by either allele alone and is analogous to heterosis which is normally effected within the cell.

Other cell-diffusible genes of this type are the  $Y$  endosperm color genes. In these cases there is no darkening of the border cells containing the dominant allele, only a gradual diminution of color from yellow to colorless. Some of the chlorophyll-controlling genes of the zebra pattern type are probably cell-diffusible. The margins of these bands of lighter green chlorophyll running across the leaf are usually indistinct. However, these have not been observed in areas where the dominant gene has been removed in somatic tissue. Many of the chlorophyll genes are cell-limited as shown by the well-defined stripes of green and white tissue in the leaves running lengthwise. These differently colored areas are produced normally

in the recessive condition but there are also cases where the colorless areas result from the removal of the dominant allele by chromosome aberration or by mutation and the margins are usually distinct.

The pericarp (*P*) and the plant color factor (*B*) are also cell-limited genes. Endosperm genes controlling the reserve food formation such as sugary (*su*), waxy (*wx*) and brittle (*bt*) are cell-limited. Adjoining cells show clear-cut effects of the dominant or recessive allele with no gradation either way. The shrunken (*sh*) condition does not appear in small recessive areas and this may be a cell-diffusible gene. Dull, floury, mealy, opaque and many defective genes have not been observed in adjoining dominant and recessive areas. Many seeds heterozygous for miniature and other defective genes have been examined and no recessive areas have been found. Either these genes are cell-diffusible or cell-lethal.

Cell-diffusible genes of the *A* type furnish an excellent example of a gene-substrate interaction. Since the gene products are transferred from cell to cell they are capable of extraction and analysis as shown by Sando and others.<sup>4</sup> Working with purple husked maize, the purple color dependent upon the presence of the dominant *A* gene, they obtained evidence for the conversion of flavanols to anthocyanidins by reduction of their corresponding homologous glucosides.

It has been shown by Clark<sup>5</sup> that the genes controlling the development of the male gametophyte in maize operate normally when separated in the cell into a number of small nuclei, provided a full complement of chromosomes is present. This evidence, together with the facts reported here, shows that gene products are effective outside the nucleus and that the interaction between different genes and different alleles of the same gene takes place in the cytoplasm in some cases.

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*THE INFLUENCE OF X-RAYS AND NEAR INFRA-RED RAYS ON  
RECESSIVE LETHALS IN DROSOPHILA MELANOGASTER\**

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The study here reported concerns the effect of supplementary treatment with near infra-red radiation on the frequency with which x-ray-induced recessive lethal mutations are produced in *Drosophila melanogaster*. Earlier work had shown that when near infra-red rays ( $\lambda$  ca. 10,000 Å) are used prior to x-rays in the treatment of the spermatozoa of *D. melanogaster*, a marked increase occurs in the frequency of detectable chromosomal rearrangements over that of controls receiving only the dose of x-rays. On the other hand, the percentage of dominant lethals is not significantly higher when near infra-red treatment precedes the x-rays than when x-rays alone are used.<sup>1</sup> We have now collected additional data which indicate that such supplementary treatment has no significant effect in this species on the frequency of production by x-rays of sex-linked recessive lethals.<sup>2</sup>

Experimental procedures involved the use of the *ClB* method for detection and verification of X-chromosome lethals. Irradiated males were mated with females of the constitution *ClB v/ec ct<sup>6</sup> v g<sup>3</sup>; Cy/Pm*. The maintenance of *Pm* in the stock, and the selection of *F*<sub>1</sub> females carrying this marker, readily permitted the detection of the non-disjunctional *XXY* exceptions, because of the suppressing effect of the Y-chromosome on eye-color variegation, and facilitated thereby the diagnosis and scoring of the *F*<sub>2</sub> cultures.

Males of the Swedish-*b*<sup>6</sup> stock were used; they were obtained from cultures derived from a single pair of flies that had been tested cytologically to insure freedom from chromosomal aberrations. The males were selected three to five days after their emergence and divided into two groups of approximately equal numbers. One of these groups was exposed to near infra-red radiation (as described by Kaufmann, Hollaender and Gay, 1946)<sup>1</sup> for a period of 48 hours either preceding (pretreatment) or following (post-treatment) exposure to x-rays. During this two-day period the group of males that represented the "controls" was kept at a temperature of 18°C. Both groups were exposed simultaneously to a 3000-roentgen dose of x-rays in capsules lying side by side. Following the combined treatment, the males were mated with *ClB* females and allowed to remain in culture bottles for three days before being discarded. This relatively short mating period was chosen as a standard in order to permit determi-

nation of the effect of post-treatment, as well as that of pretreatment, since in the span of three days the accelerating action of near infra-red radiation on the progress of spermatogenesis (detected in an earlier study)<sup>1</sup> is not sufficient to make sperm that was immature at the time of x-ray treatment available for transfer in copulation.

*Results.*—The lethal-mutation rates determined by these tests are indicated in table 1. Males exposed to near infra-red radiation alone provided only 4 lethals among 2316 spermatozoa tested. The percentage of mutations ( $0.17 \pm 0.08$ ) is not significantly different from that occurring

TABLE 1  
LETHAL MUTATION RATE (CIB TESTS) AMONG SPERMATOZOA OF MALES EXPOSED TO X-RAYS OR TO X-RAYS PLUS NEAR INFRA-RED RAYS

TYPE OF TREATMENT: X-RAY IN ROENTGENS, NEAR INFRA-RED IN HOURS	NUMBER SPERMS TESTED	LETHAL MUTATIONS	PER CENT MUTATIONS
NIR 48 hrs.	2316	4	$0.17 \pm 0.08$
X-ray 3000 r	3393	253	$7.46 \pm 0.45$
3000 r + 48 hrs.	1989	145	$7.29 \pm 0.58$
48 hrs. + 3000 r	1770	124	$7.01 \pm 0.61$

among spermatozoa of one- to two-day-old males of the *Sw-b* stock at 22°C.<sup>8</sup> Near infra-red radiation in itself, therefore, does not appear to be effective in inducing the types of change that are represented among the group of recessive lethals. Nor does this type of radiation when used prior to or subsequent to a 3000-r dose of x-rays modify to an appreciable extent the frequency of induced lethal mutations, since, as table 1 indicates, samples of the pretreatment and post-treatment series and the controls all yielded about 7 to 7.5% of lethals. (The difference between this frequency and the 8 to 8.7% reported for the 3000-r dosage level by other workers may be attributable in part to differences in the stocks used, or to differences in dosimetry.)

In order to appraise these findings, an effort was made to determine the frequency of occurrence of chromosomal rearrangements among the group of recessive lethals. Previous studies, utilizing salivary-gland-chromosome preparations, had revealed that in some cases there is no detectable chromosomal alteration at the locus of the induced lethal, whereas in others there is a deficiency of one or more bands, or involvement in a gross rearrangement, often without any visible deletion.<sup>4-7</sup> The proportion of the lethals associated with chromosomal rearrangements has been determined in a series of experiments;<sup>4, 8-11</sup> it appears, on the basis of the limited data available, to vary with the x-ray dose, but at the 3000-r level is of the order of magnitude of 35% (summarized data are given by Lea and Catcheside).<sup>6</sup> Comparable values were obtained for 100 of the 526 lethal mutations detected in our experiments. The 100,

selected at random in equal numbers from the combination-treatment series and the controls, were analyzed by the salivary-gland-chromosome method. Among 50 derived from the combination treatment, 18, or 36%, showed gross rearrangements involving the X-chromosome; 11 were found among 25 lethals examined in the pretreatment series, and 7 among 25 in the post-treatment series. In the control group, 14 out of 50, or 28%, revealed X-chromosome rearrangements. The aberrations were of the types that are customarily detected by salivary-gland-chromosome analysis following treatment of males with a 3000-r dose of x-rays, and included large deficiencies, transpositions, inversions, reciprocal translocations, and complex rearrangements involving two or more chromosomes. We have not carried out the extensive series of genetic tests that would be required to determine the precise location of the lethal mutation with respect to the points of breakage involved in each rearrangement; but, in the light of Demerec's finding<sup>4</sup> that a breakage point coincided with the locus of the lethal in 24 of 26 cases studied (92.3%), it appears that a similar correlation may obtain in our material.

*Discussion.*—The data and the considerations here presented indicate, therefore, that a considerable fraction of the lethal mutations induced in our experiments is associated with gross structural changes in the chromosomes. Rearrangements of the types represented had been found in an earlier experiment to increase in frequency about 50% when treatment of the spermatozoa with near infra-red radiation preceded a 4000-r dose of x-rays.<sup>1</sup> However, we have not found a corresponding rise in the frequency of recessive lethals when such combination treatment is applied. This suggests that the lethals associated with gross chromosomal alterations are not dependent for their expression on the production of rearrangements. If they were, pretreatment with near infra-red radiation should effect an increase of about 1 to 1.5% in the frequency of recessive lethals over that in the x-ray controls.

Analysis of dose-frequency relations determined experimentally in studies of lethals and chromosomal aberrations had previously led Lea and Catchside<sup>6</sup> to formulate a detailed theory based on the alternative assumption that radiation-induced recessive lethals and chromosomal rearrangements in *Drosophila* result independently from a single type of primary effect. This interpretation has also been formulated by Herskowitz.<sup>11</sup> Fano,<sup>12</sup> however, in a recent note, has pointed out that the consequences of this alternative assumption also are at variance with the total experimental evidence. The evidence now available from the near infra-red experiments makes it seem reasonably certain that the negative portion of the theory advanced by Lea and Catchside concerning the origin of recessive lethals is essentially correct—namely, that the lethals associated with chromosomal rearrangements in *Drosophila* do not repre-



sent a special class caused by position effect and requiring two ionizing particles for their production.

In the absence of any acceptable comprehensive theory of the mechanism of induction of lethals and of viable rearrangements, it may still be useful to compare the values obtained experimentally following the combination treatment with theoretical estimates derived from alternative assumptions. These estimates, supplied by Dr. U. Fano, were based on the methods developed in his previous note;<sup>13</sup> the pertinent calculations will be published in Year Book No. 46 of the Carnegie Institution of Washington. In making the estimates it has been assumed tentatively (in agreement with the data obtained at 4000 r) that the frequency of cytologically detectable X-chromosome breaks induced by an x-ray dose within the range from 2000 r to 4000 r will be increased by 50% under the influence of infra-red treatment. The infra-red treatment should then have the following effects:

1. The frequency of sex-linked recessive lethals at 3000 r should increase by about 17% if  $\frac{1}{3}$  of those lethals were due to position effect, and decrease by about 5% or more according to the Lea-Catcheside hypothesis. Our experimental results show a decrease of this order of magnitude (table 1, line 4 compared with line 2), although the difference is not statistically significant.

2. The fraction of sex-linked recessive lethals associated with rearrangements should increase from approximately  $\frac{1}{8}$  to  $\frac{3}{7}$  according to the position-effect hypothesis, and to over  $\frac{1}{2}$  according to the Lea-Catcheside hypothesis. Our experimental data show an over-all increase from 28 to 36%, which is more nearly comparable in magnitude with that expected on the former hypothesis than on the latter. It should be pointed out, however, that in these experiments the number of cases analyzed is small, and the errors correspondingly large ( $28 \pm 6\%$ , and  $36 \pm 7\%$ ). Moreover, among the group of lethals induced by x-rays following pretreatment with near infra-red radiation—the type of experiment in which the 50% increase in frequency of viable chromosomal rearrangements was effected—there were 11 lethals associated with chromosomal rearrangements out of 25 examined, a frequency of  $44 \pm 10\%$ ; and this value, although not statistically significant, might be reconciled with the Lea-Catcheside hypothesis.

3. The fraction of eggs hatching when the spermatozoa used in their fertilization had been exposed to 2000 roentgens of x-rays should be reduced by not less than about 10% (of the fraction itself), according to either hypothesis. Our actual counts<sup>1</sup> correspond closely to this expected value, since 51.4% of the eggs hatched when sperms were treated with 2000 roentgens of x-rays, and only 46.7% when treatment with near infra-red radiation preceded the x-rays.



It is obvious, therefore, that the application of this type of analysis to the formulation of a general theory of the origin of lethals and chromosome rearrangements will require more extensive data than have been provided in the present paper. Further pertinent data might also be obtained from a study of frequency of the various types of change induced by x-rays at different dosage levels following pretreatment with near infra-red radiation.

The experimental data here presented, in conjunction with those obtained in earlier experiments,<sup>1, 14</sup> permit a more comprehensive view than was previously possible of the action of near infra-red radiation in modifying the frequency of x-ray-induced chromosomal rearrangements in *Drosophila*. This "sensitizing" action now appears to apply to the production of viable chromosomal rearrangements, which are detected by salivary-gland-chromosome analysis, and presumably also to their inviable counterparts, the multiple-break type of lethals that lead to death in embryonic stages of the individuals carrying them. Supplementary treatment with near infra-red radiation did not effect any significant increase in the frequency of the single-break type of dominant lethal, or of sex-linked recessive lethals. These findings, together with our observations that near infra-red radiation in itself is ineffective in inducing either lethal mutations or chromosomal rearrangements, suggest that this agent is not responsible for initiating or producing breakage of chromosomes. As was pointed out in our earlier publication,<sup>1</sup> near infra-red radiation of wave-length 10,000 Å provides only about 1.2 electron volts wherever a quantum is absorbed, and this amount is not as a rule sufficient to break chemical bonds. Under these conditions the production of primary breaks would be a function of the x-ray dose alone, even in treatments that combine near infra-red and x-rays. In appraising this interpretation, consideration must be given to the finding of Swanson and Hollaender<sup>12</sup> that treatment of microspores of *Tradescantia* with near infra-red rays following their exposure to x-rays produces a significant increase in chromosome breakage beyond that found in the controls. In order to relate to a single mechanism the modifying action of near infra-red radiation when used prior to or subsequent to x-rays, these authors have suggested that the chromosome structure may be weakened by either type of radiation and that the supplementary action of the other may then be effective in producing a thoroughgoing break. This interpretation attributes the increased frequency in detectable chromatid breaks to an increase in the number of primary breaks. Since we have not found evidence in our experiments with *Drosophila* to support this assumption, we are inclined to the point of view indicated in our earlier publication<sup>1</sup>—that near infra-red radiation acts as a "sensitizing" agent by increasing the number of breaks available for participation in the production of new chromosomal rearrangements. This increase could be realized if recombination were

facilitated at the expense of restitution. Such a mechanism would account for the fact that in *Tradescantia* the frequency of double deletions, which presumably are realized immediately following the production of the lesion by x-rays, is increased by pretreatment but not by post-treatment with near infra-red, whereas single deletions and interchromosomal exchanges are increased in frequency both by pretreatment and by post-treatment. We have not completed a detailed analysis of the effect on the chromosomes of the x-rayed spermatozoan of *Drosophila* of exposure to a dose of near infra-red radiation equal to that used in the pretreatment experiments; but exposure for shorter intervals of eggs fertilized by x-rayed sperm has elicited chromosomal rearrangements with a frequency in excess of that obtained in controls kept at 18° to 28°C.<sup>14</sup> Facilitation of chromosome movement may have been an important factor in promoting recombination in these cases, since the eggs were exposed during the period of syngamy and early cleavage, when the potential breaks first become available for participation in the formation of rearrangements. On the other hand, the pronounced effect of near infra-red radiation in the pretreatment experiments emphasizes the possibility that this agent may produce such changes in the materials of the chromosomes that the process by which restitution is normally effected is either inhibited or delayed, thereby making additional breaks available for the formation of new combinations.

The more precise definition of the mode of action of near infra-red radiation that is now possible opens the way for an attack on questions relating to its effect on specific cellular components; it also emphasizes the potentialities of this agent as a tool in experiments designed to modify the recombination phase of the process of induced structural change.

*Summary.*—Supplementary treatment of the spermatozoa of *D. melanogaster* with near infra-red radiation does not effect any significant increase in the frequency of production by x-rays of recessive, sex-linked lethal mutations. Analysis of a sample of 100 of these lethals by the salivary-gland-chromosome method revealed that 32% were associated with gross chromosomal alterations. A consideration of these data and those obtained in previous experiments suggests that radiation-induced recessive lethals are not attributable primarily to a position effect dependent on the establishment of new associations by the gene concerned. Consideration of the combined data also suggests that near infra-red radiation, when used as a supplementary treatment, is effective in increasing the frequency of chromosomal rearrangements by facilitating recombination, presumably at the expense of restitution, among the ends of chromosomes broken by the ionizing radiation.

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## ON INFINITE COMPLEXES WITH AUTOMORPHISMS\*

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When in an infinite complex  $K$  a group  $G$  of automorphisms operates without fixed cells and with finite fundamental domain, then there exist purely algebraic relations between certain homology properties of  $K$  and the abstract structure of  $G$ . This is established in the present note as an application of a previous general result,<sup>1</sup> and geometric examples are investigated, concerning, in particular, coverings of closed manifolds and their homotopy groups.

1. Let  $K$  be a closure finite complex in the sense of combinatorial topology; its finite integer chain groups will be denoted by  $C_n$ , the boundary homomorphism of  $C_n$  into  $C_{n-1}$  by  $\partial$ ,  $n \geq 0$  ( $C_{-1}$  is the additive group of integers, and the boundary of a 0-cell  $c_0$  is defined by  $\partial c_0 = 1$ ), and the homology groups based upon finite integer chains by  $H_n$ . If  $H_n = 0$ ,  $K$  is said to be *acyclic* in the dimension  $n$ ; when  $K$  is a geometric complex,  $H_0 = 0$  means that it is connected.

For a given Abelian coefficient group  $J$ , an  $n$ -cochain  $f^n$  in  $K$ ,  $n \geq 0$ , is a  $J$ -valued function of the  $n$ -cells  $c_n$  of  $K$ , or a homomorphism of  $C_n$  into  $J$ ; its coboundary  $\delta f^n$  is the  $(n+1)$ -cochain defined by  $\delta f^n(a_{n+1}) =$

$f^n(\partial a_{n+1})$  for all  $a_{n+1} \in C_{n+1}$ . The group of all  $n$ -cochains is denoted by  $C^n$ ;  $\delta$  is a homomorphism of  $C^n$  into  $C^{n+1}$ , with kernel  $Z^n$ , and with  $\delta\delta = 0$ .  $H^n = Z^n/\delta C^{n-1}$  is the  $n$ th ordinary cohomology group of  $K$ ,  $n \geq 0$  (by  $\delta C^{-1}$  we understand 0).

A cochain  $f^n$  is called *finite*, if  $f^n(c_n) \neq 0$  for only a finite number of  $n$ -cells  $c_n$  of  $K$ ; the finite  $n$ -cochains form a subgroup  $\mathcal{C}^n$  of  $C^n$ . If  $K$  is also star finite—i.e., if the coboundary of a finite cochain is always finite—, then the groups  $C^n$  lead to “finite” cohomology groups  $\mathcal{H}^n$  of  $K$ ,  $n \geq 0$ . By  $(\mathcal{H}^n)_0$  we denote the subgroup of  $\mathcal{H}^n$  defined by those finite cocycles, which are coboundaries of arbitrary cochains  $\in C^{n-1}$  (i.e.,  $(\mathcal{H}^n)_0$  in the kernel of the natural injection of  $\mathcal{H}^n$  into  $H^n$ ).

2. Now we suppose that  $K$  is a “complex with automorphisms”; this means that a group  $G$  of automorphisms of  $K$  without fixed cells is given. In other words,  $K$  is a regular covering of a complex  $\mathfrak{K}$  and  $G$  the corresponding group of covering transformations. The set of all cells  $xc_n$ ,  $x \in G$ , for a certain cell  $c_n$  of  $K$ , is called a *transitivity domain* of  $G$ ; there is a one-one correspondence between the cells of  $K$  and the transitivity domains of  $G$  in  $K$ .

A cochain  $f^n$  of  $K$  will be called *G-finite*, if it is finite on each transitivity domain; i.e., if, for each cell  $c_n$ ,  $f^n(xc_n) \neq 0$  for only a finite number of elements  $x \in G$ . A function of  $x \in G$  with that property will be said in short to be “almost 0.” When  $f^n$  is *G-finite*, then  $f^n(xa_n)$  is almost 0 for any given chain  $a_n$ , and it follows that  $\delta f^n(xa_{n+1}) = f^n(x\partial a_{n+1})$  is almost 0 for any given  $(n+1)$ -chain  $a_{n+1}$ ; i.e., that the coboundary of a *G-finite* cochain is again *G-finite*. Hence the *G-finite* cochains lead to *G-finite cohomology groups*.

In a previous note<sup>1</sup> I introduced in a complex with automorphisms, for a given group  $\psi$  of  $J$ -valued functions of  $x \in G$ ,  $\psi$ -cohomology groups  $H_\psi^n$ ,  $n \geq 0$ ; they are defined by means of cochains which on each transitivity domain are elements of  $\psi$ . When  $\psi$  is the group of all almost 0 functions from  $G$  to  $J$ , these  $H_\psi^n$  are obviously identical with the *G-finite* cohomology groups of  $K$  defined above. We shall use here the notations and results of [1], for that special  $\psi$  only;  $H_\psi^n$  will denote the *G-finite* cohomology groups of  $K$ ,  $(H_\psi^n)_0$  the subgroup of  $H_\psi^n$  consisting of those *G-finite* cohomology classes which are in the 0-class of  $H^n$  (the kernel of the injection of  $H_\psi^n$  into  $H^n$ ).

3. Let  $G$  be an arbitrary group. We consider  $J$ -valued functions  $f^n(x_0, x_1, \dots, x_n)$  of  $n+1$  variables  $\in G$ ,  $n \geq 0$ , with the property that for any given  $x_0, x_1, \dots, x_n$  the function  $f^n(xx_0, xx_1, \dots, xx_n)$  of  $x \in G$  is almost 0. By  $\delta f^n$  we understand the function of  $n+2$  variables  $\in G$  defined by

$$\delta f^n(x_0, x_1, \dots, x_{n+1}) = \sum_{i=0}^{n+1} (-1)^i f^n(x_0, \dots, x_{i-1}, x_{i+1}, \dots, x_{n+1}),$$

which obviously has the same property. Let  $F^n$  be the group of all these functions

$f^n, n \geq 0$ .  $\delta$  is a homomorphism of  $F^n$  into  $F^{n+1}$ , with kernel  $F_0^n$ , and with  $\delta\delta = 0$ . The "cohomology groups"  $F_0^n/\delta F^{n-1}$ ,  $n \geq 0$  (by  $\delta F^{-1}$  we understand 0) will be denoted by  $\Pi^n(G, J)$ .

These groups  $\Pi^n(G, J)$ , associated with  $G$  and  $J$  in a purely algebraic way, may be considered as the  $G$ -finite cohomology groups of a certain abstract complex  $K_\sigma$  with the automorphism group  $G$  (cf. [1], §5; with the notation used there, they had to be denoted by  $\Gamma_\psi^n(G, J)$ ,  $\psi$  being again the group of all almost 0 functions from  $G$  to  $J$ ).

4. We return to the complex  $K$  with the automorphism group  $G$  considered in §2, and we suppose that  $K$  is acyclic in all dimensions  $< N$  ( $N$  being a positive integer). E.g., if  $K$  is a connected geometric complex, this holds for  $N = 1$ , if  $K$  is simply connected for  $N = 2$ . From the general result of [1], §8, we deduce the following isomorphisms for the  $G$ -finite cohomology groups  $H_\psi^n$  of  $K$ :

$$(4.1) \quad \begin{aligned} H_\psi^n &\cong \Pi^n(G, J), & n < N, \\ (H_\psi^N)_0 &\cong \Pi^N(G, J). \end{aligned}$$

This means, roughly speaking, that in an acyclic complex with automorphisms the  $G$ -finite cohomology groups are determined by the abstract structure of the automorphism group  $G$  (and by  $J$ ) and are given by the explicit description in §3.

5. Now we assume further that  $G$  has in  $K$  a "finite fundamental domain." This means that in each dimension the number of transitivity domains is finite; in other words, that  $K$  is a covering of a finite complex  $\mathcal{R}$  and  $G$  the corresponding covering transformation group. In this case a  $G$ -finite cochain is the same as a finite one, and  $H_\psi^n$  is identical with the  $n$ th cohomology group of  $K$  based upon finite cochains, denoted in §1 by  $\mathcal{H}^n$ ;  $(H_\psi^n)_0$  is the group  $(\mathcal{H}^n)_0$ . These groups  $\mathcal{H}^n$  and  $(\mathcal{H}^n)_0$  are, of course, defined in  $K$  independently of any automorphism group. But from the above result (4.1) it follows:

**THEOREM (5.1):** *Let  $K$  be a complex which is acyclic in all dimensions  $< N$ , and  $G$  a group of automorphisms of  $K$  without fixed cells and with finite fundamental domain. Then  $\mathcal{H}^n \cong \Pi^n(G, J)$ ,  $n < N$ , and  $(\mathcal{H}^N)_0 \cong \Pi^N(G, J)$ .*

This is the main result of this note. It states, for example, that for different automorphism groups  $G$  operating in the same complex, the conditions of the theorem being fulfilled, the groups  $\Pi^n(G, J)$  are isomorphic; and that, when the same group  $G$  operates in different complexes, their groups  $\mathcal{H}^n$  must be isomorphic.

The groups  $\mathcal{H}^n$  and  $(\mathcal{H}^n)_0$  of an infinite polytope appear to have a geometric meaning, in connection with its "end-points" in the sense of Freudenthal and Hopf;<sup>3</sup> this will be studied elsewhere. Further results can be obtained in terms of the residual cohomology groups of the cohomology



sequence (relative to  $\psi$ ; cf. [1], §3). We give here only two immediate applications of the theorem (5.1); the result of the second is well known.

6. Let  $\mathfrak{M}$  be a closed orientable manifold of dimension  $m$ , aspherical in all dimension  $n$ ,  $1 < n < N$  (i.e., a continuous map of an  $n$ -dimensional sphere into  $\mathfrak{M}$  is always nullhomotopic, for these  $n$ ; or the homotopy groups of  $\mathfrak{M}$ ,  $\pi_n(\mathfrak{M})$ , are  $= 0$ ,  $1 < n < N$ ). Let  $G$  be the fundamental group of  $\mathfrak{M}$ ,  $M$  its universal covering. Then the following isomorphisms hold for the homology groups of  $M$ :

$$(6.1) \quad H_n(M) \cong \Pi^{m-n}(G, J), \quad n > m - N,$$

$J$  being the group of integers. For by the duality theorem for manifolds  $H_n(M) \cong \mathcal{H}^{m-n}(M)$ , and (6.1) follows from (5.1). If in particular  $N > \frac{m}{2}$ , (6.1) holds for  $n = N$ . Since  $\pi_n(M) \cong \pi_n(\mathfrak{M}) = 0$ ,  $1 < n < N$ , and  $M$  is simply connected, then by a theorem of Hurewicz<sup>3</sup>  $H_N(M)$  is isomorphic to  $\pi_N(M)$ , which in turn is isomorphic to  $\pi_N(\mathfrak{M})$ . Hence

$$(6.2) \quad \pi_N(\mathfrak{M}) \cong \Pi^{m-N}(G, J),$$

$J$  being always the group of integers: *If  $\mathfrak{M}$  is a closed orientable manifold of dimension  $m$ ,  $N$  an integer with  $\frac{m}{2} < N \leq m$ , and if  $\pi_n(\mathfrak{M}) = 0$  for  $1 < n < N$ , then  $\pi_N(\mathfrak{M})$  is determined by the fundamental group  $G$  of  $\mathfrak{M}$  and given explicitly as the group  $\Pi^{m-N}(G, J)$ .*

In a closed orientable 3-dimensional manifold  $\mathfrak{M}$ , for example, we have always  $\pi_2(\mathfrak{M}) \cong \Pi^1(G, J)$ . This result has already been established by Specker (in an as yet unpublished paper), who introduced, for the same purpose, a group isomorphic to our  $\Pi^1(G, J)$ .

7. Let  $M$  be an open orientable manifold of dimension  $m$ , acyclic in all dimensions,  $G$  an automorphism group of a cell subdivision of  $M$ , without fixed cells and with finite fundamental domain. Since, for any coefficient group  $J$ ,  $\mathcal{H}^n = 0$  for  $n > m$ , and by the duality theorem  $\mathcal{H}^m = J$ ,  $\mathcal{H}^n = 0$  for  $n < m$ , it follows from (5.1) that  $\Pi^n(G, J) = 0$  for all  $n$  except for  $n = m$ , where  $\Pi^m(G, J) \cong J$ . Hence there exists an algebraic relation between the dimension of  $M$  and the group  $G$ : *It is impossible to have in two open acyclic manifolds of different dimensions isomorphic automorphism groups without fixed cells and with finite fundamental domain.*<sup>4</sup>

**Examples:**<sup>4</sup> (1) If two closed manifolds both have a Euclidean space as universal covering, and if their fundamental groups are isomorphic, then they are of the same dimension. (2) A free Abelian automorphism group (without fixed cells and with finite fundamental domain) of an open acyclic manifold of dimension  $m$  is of rank  $m$ .



The results of the whole note can be generalized, when homology groups and acyclicity with other coefficients than integers are considered.

\* Presented to the American Mathematical Society, April, 1947.

<sup>1</sup> Eckmann, B., these PROCEEDINGS, **33**, 275-281 (1947). This note is referred to in the text by [1].

<sup>2</sup> Freudenthal, H., *Math. Zeitschrift*, **33**, 692-713 (1931); Hopf, H., *Comment. Math. Helv.*, **16**, 81-100 (1943/44).

<sup>3</sup> Hurewicz, W., *Proc. Akad. Amsterdam*, **38**, 521-528 (1935), Theorem I.

<sup>4</sup> Cf. Hurewicz, W., *Ibid.*, **39**, 215-224 (1936), Section 6.

## AN OSCILLATION THEOREM FOR CONTINUOUS SPECTRA

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Let  $p = p(t)$ ,  $q = q(t)$  be two continuous functions on the half-line  $0 \leq t < \infty$  and let  $p > 0$ . Suppose that the differential equation  $(px')' + qx = 0$  does not possess two linearly independent solutions  $x = x(t)$  of class  $(L^2)$ , i.e., that some solution is not contained in the Hilbert space

$$\int_0^\infty x^2(t) dt < \infty \quad (1)$$

(without loss of generality, all solutions can be assumed to be real). The restriction thus imposed on the differential equation is satisfied if and only if a boundary condition, such as  $x(0) = 0$  or  $x'(0) = 0$  and generally

$$x(0) \cos \phi + x'(0) \sin \phi = 0, \quad (2)$$

determines for

$$(px')' + (q + \lambda)x = 0 \quad (3)$$

an eigenwert problem.<sup>1</sup>

It will be shown that, if the coefficient functions of (3) are fixed, there belongs to every point  $\lambda$  of the line  $-\infty < \lambda < \infty$  at least one boundary condition (2) in such a way that  $\lambda$  becomes a point of the spectrum of the eigenwert problem (2), (3). In the terminology of F. Klein, this can be interpreted as representing a general "oscillation theorem" of the Heine-Stieltjes type,<sup>2</sup> in the following sense:

If the coefficient functions of (3) are fixed, every choice of (2) determines a spectrum  $S(\phi)$ , containing the point spectrum  $P(\phi)$  (the latter can be vacuous). The derivative of the set  $S(\phi)$  consists of the continuous spectrum and of the cluster points of  $P(\phi)$ . According to Weyl,<sup>3</sup> this derivative is independent of  $\phi$  and can, therefore, be denoted simply by  $S'$ . On

the other hand,  $S(\phi)$ , hence  $P(\phi)$ , will vary with  $\phi$ . Hence, what the theorem to be proved actually states is that, *when  $\phi$  varies, every  $\lambda$  not contained in the invariant set  $S'$  must occur in the point spectrum belonging to some  $\phi$* ; as a matter of fact, that the same is true if only those points of the point spectra are considered which are neither in the continuous spectrum nor among the cluster points of the point spectrum belonging to a fixed  $\phi$ . In other words, if  $R$  denotes the complement of  $S'$  (so that  $R$  is an invariant set of  $\lambda$ -values which is open, possibly vacuous), then points of the point spectra  $P(\phi)$  will sweep through each of the open intervals (including half-lines) which constitute the invariant set  $R$ . It remains undecided whether, under reasonable restrictions, this movement of  $P(\phi)$  over  $R$  must or need not possess that property of monotony which is suggested by the limiting case of classical oscillation theorems;<sup>4</sup> a case in which  $R$  degenerates into the entire  $\lambda$ -line.

It follows from Weyl's theory<sup>5</sup> that a given  $\lambda$  is not in  $S(\phi)$  if and only if, corresponding to every continuous  $g(t)$  of class  $(L^2)$ , the inhomogeneous differential equation

$$(px')' + (q + \lambda)x = g \quad (4)$$

and the boundary condition (2) possess a (unique) solution  $x(t)$  satisfying (1). Hence, the italicized assertion is equivalent to the statement that there belong to every  $\lambda$  some  $\phi$  and some continuous  $g(t)$  of class  $(L^2)$  in such a way that there does not exist any  $x(t)$  satisfying (4), (2), (1). The proof will be based on this re-wording of the theorem.

It can be assumed that (3) has no non-trivial solution satisfying (1). For otherwise  $\lambda$  is in  $P(\phi)$  for some  $\phi$ , and therefore in the corresponding  $S(\phi)$ . Accordingly, the set  $S'$  being invariant, the assertion becomes that, if  $\lambda$  and  $\phi$  are arbitrarily fixed, the three requirements (4), (2), (1) fail to possess a solution  $x(t)$  for *some* continuous  $g(t)$  of class  $(L^2)$ .

Let  $\lambda$  and  $\phi$  be arbitrarily fixed and let  $x = y(t)$  be a non-trivial solution of (3) and (2) (such functions  $y(t)$  exist, since (1) is not required of  $x = y$ ). Since  $y(t)$  does not vanish identically, there exists a positive  $t_0$  for which  $y(t_0) \neq 0$ . With reference to such a  $t_0$ , consider on the interval  $0 \leq t \leq t_0$  the boundary value problem

$$z(0) \cos \phi + z'(0) \sin \phi = 0, \quad z(t_0) = 0 \quad (5)$$

for the differential equation

$$(pz')' + (q + \lambda + \mu)z = 0, \quad (6)$$

where  $\lambda$  denotes the fixed value to which  $y(t)$  belongs. Since (6) and the pair of conditions (5) represent a regular Sturm-Liouville problem, there exist characteristic values  $\mu$ , i.e., values corresponding to which (6) and (5) have a non-trivial solution  $z(t)$ , where  $0 \leq t \leq t_0$ . It should be noted

that, since  $z(t)$  does not vanish identically, the second of the conditions (5) prevents the vanishing of  $z'(t_0)$ . Accordingly  $t_0$  satisfies the following pair of conditions:

$$y(t_0) \neq 0, \quad z'(t_0) \neq 0. \quad (7)$$

After  $\lambda, \phi, y(t), t_0, \mu, z(t)$  have been fixed, define for  $0 \leq t < \infty$  a function  $g(t)$  by placing

$$g = -\mu z \text{ if } 0 \leq t < t_0 \text{ and } g = 0 \text{ if } t_0 \leq t < \infty. \quad (8)$$

In view of the second of the conditions (5), this  $g(t)$  remains continuous at  $t = t_0$ . Furthermore,  $g(t)$  is of class  $(L^2)$ , by the second part of (8). It will be shown that this  $g(t)$  has the property needed for the completion of the proof.

Suppose that  $g(t)$  fails to have the property in question. Then  $g(t)$  is such that there exists on the half-line  $0 \leq t < \infty$  a function  $x(t)$  satisfying (4), (2), (1). It will be shown that the assumption of such an  $x(t)$  leads to a contradiction.

First, if  $t_0 \leq t < \infty$ , then (4) reduces to (3)<sup>1</sup> by the second part of (8). Hence,  $x(t)$  satisfies (3) for  $t_0 \leq t < \infty$ . Since  $x(t)$  satisfies (1) also, and since (3) is supposed to have no non-trivial solution satisfying (1), it follows that  $x(t) = 0$  for  $t_0 \leq t < \infty$  (otherwise a non-trivial solution of (3) and (1) would be represented by the function which is identical with  $x(t)$  for  $t_0 \leq t < \infty$  and is defined by the initial conditions  $x(t_0), x'(t_0)$  for  $0 \leq t < t_0$ ). But  $x(t)$  is a solution of (4) for  $0 \leq t < \infty$  and must therefore be differentiable at  $t = t_0$ . Hence, the identical vanishing of  $x(t)$  for  $t_0 \leq t < \infty$  implies that

$$x(t_0) = 0, \quad x'(t_0) = 0. \quad (9)$$

Next, (5), (6) and the first part of (8) show that  $x = z(t)$  satisfies (2) and is a solution of (4) for  $0 \leq t \leq t_0$ . Hence  $x(t)$ , being a solution of (2) and (4) for  $0 \leq t < \infty$ , must be of the form

$$x(t) = z(t) + cy(t) \text{ for } 0 \leq t \leq t_0, \quad (10)$$

where  $y(t)$  denotes that solution of (3) and (2) which has been chosen before (5), and the  $c$  occurring in (10) is an appropriate constant.

If (10) is differentiated at  $t = t_0$ , it follows from (9) that both  $z + cy$  and  $z' + cy'$  vanish at  $t = t_0$ . Since this represents two homogeneous linear equations for  $1, c$ , the determinant  $yz' - zy'$  must vanish at  $t = t_0$ . In view of the second of the conditions (5), this means the vanishing of  $yz'$  at  $t = t_0$ . Since this contradicts (7), the proof is complete.

<sup>1</sup> This criterion is equivalent to the fact on which the alternative of *Grenzpunktfall* and *Grenzkreisfall* has been based by H. Weyl, *Math. Ann.*, **68**, 220-269 (1910) (theorem 5 on p. 238).

<sup>1</sup> Cf. M. Bôcher, *Enc. der math. Wiss.*, Vol. 2, Part 1, 1900, pp. 450–457.

<sup>2</sup> *Loc. cit.*, Vol. 1, pp. 251–252.

<sup>3</sup> Cf. *loc. cit.*, Vol. 2, pp. 443–444 (Sturm) and pp. 453–455 (Lamé).

<sup>4</sup> *Loc. cit.*, Vol. 1, p. 251.

## ON THE COMPOSITION OF QUADRATIC FORMS

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In a recent paper Dubisch<sup>1</sup> has obtained some essentially new results on the problem of composition of quadratic forms. On the basis of these results I derive others by orienting the solution of the problem in the opposite direction. In this way the solution appears simpler and more unified.

Let  $g(u)$  be a real non-singular *indefinite* quadratic form of index  $t$  in  $p$  variables  $u_1, \dots, u_p$ . Thus  $0 < t < p$ . A (slightly special) case of the Hurwitz problem<sup>2</sup> in the real is to find a real non-singular quadratic form  $f(x)$  in  $n$  variables  $x_1, \dots, x_n$ , such that

$$g(u)f(x) = f(y)$$

where  $y_1, \dots, y_n$  on the right are real bilinear functions of  $u_1, \dots, u_p$  and  $x_1, \dots, x_n$ . It can be easily seen that the form  $f$  must have zero signature (and consequently  $n$  must be even). Furthermore, as concerns the relation between  $p$  and  $n$ , Dubisch's results may be stated as follows:

**THEOREM 1.** *The Hurwitz problem is possible in the real if and only if  $n$  is the following arithmetic function of  $p$ :*

$$\begin{aligned} \text{for } p = 2r + 1 \quad & \begin{cases} r \equiv 0 \pmod{4}, & t \equiv 0, 1 \pmod{4}: & n = \mu \cdot 2^r; \\ r \equiv 3 \pmod{4}, & t \equiv 0, 3 \pmod{4}: & n = \mu \cdot 2^r; \\ \text{otherwise} & : & n = \mu \cdot 2^{r+1}; \end{cases} \\ \text{for } p = 2r + 2 \quad & \begin{cases} r \equiv 3 \pmod{4}, & t \equiv 0 \pmod{4}: & n = \mu \cdot 2^r; \\ r \equiv 2 \pmod{4}, & t \equiv 1, 3 \pmod{4}: & n = \mu \cdot 2^{r+2}; \\ r \equiv 0 \pmod{4}, & t \equiv 3 \pmod{4}: & n = \mu \cdot 2^{r+2}; \\ \text{otherwise} & : & n = \mu \cdot 2^{r+1}; \end{cases} \end{aligned}$$

where  $\mu$  is any positive integer.

Using these results and writing

$$n = \mu \cdot 2^{4\alpha + \beta} (\mu \text{ odd}; \beta = 0, 1, 2, 3)$$

we prove

THEOREM 2. *The Hurwitz problem is possible in the real if and only if  $p$  is the following arithmetic function of  $n$ :*

$$\begin{aligned} \text{for } t \equiv 0 \pmod{4}: & \quad p \leq 8\alpha + 2^\beta; \\ \text{for } t \equiv 1 \pmod{4}: & \quad p \leq 8\alpha + 2^\beta - 3[\beta/3]; \\ \text{for } t \equiv 2 \pmod{4}: & \quad p \leq 8\alpha + 2\beta; \\ \text{for } t \equiv 3 \pmod{4}: & \quad p \leq 8\alpha + \beta^2 - 2[\beta/3]; \end{aligned}$$

where  $[\beta/3]$  denotes the integer part of  $\beta/3$ .

*Proof:* From the results of Theorem 1 we obtain, omitting the insertion of  $(\text{mod } 4)$  in the congruences for simplicity, the following:

$$\begin{aligned} t \equiv 0 & \begin{cases} p = 2r + 1 & \begin{cases} r \equiv 0, 3 & : & n = \mu \cdot 2^r; \\ r \equiv 1, 2 & : & n = \mu \cdot 2^{r+1}; \end{cases} \\ p = 2r + 2 & \begin{cases} r \equiv 3 & : & n = \mu \cdot 2^r; \\ r \equiv 0, 1, 2 & : & n = \mu \cdot 2^{r+1}; \end{cases} \end{cases} \\ t \equiv 1 & \begin{cases} p = 2r + 1 & \begin{cases} r \equiv 0 & : & n = \mu \cdot 2^r; \\ r \equiv 1, 2, 3 & : & n = \mu \cdot 2^{r+1}; \end{cases} \\ p = 2r + 2 & \begin{cases} r \equiv 2 & : & n = \mu \cdot 2^{r+2}; \\ r \equiv 0, 1, 3 & : & n = \mu \cdot 2^{r+1}; \end{cases} \end{cases} \\ t \equiv 2 & \begin{cases} p = 2r + 1 \\ p = 2r + 2 \end{cases} \text{ every } r & : & n = \mu \cdot 2^{r+1}; \\ t \equiv 3 & \begin{cases} p = 2r + 1 & \begin{cases} r \equiv 3 & : & n = \mu \cdot 2^r; \\ r \equiv 0, 1, 2 & : & n = \mu \cdot 2^{r+1}; \end{cases} \\ p = 2r + 2 & \begin{cases} r \equiv 0, 2 & : & n = \mu \cdot 2^{r+2}; \\ r \equiv 1, 3 & : & n = \mu \cdot 2^{r+1}. \end{cases} \end{cases} \end{aligned}$$

We now make  $n = \mu \cdot 2^r$  in every case: thus replace  $r$  by  $r - 1$  whenever  $n = \mu \cdot 2^{r+1}$ , and  $r$  by  $r - 2$  whenever  $n = \mu \cdot 2^{r+2}$ . In this way we obtain the following possible cases for  $n = \mu \cdot 2^r$ :

$$\begin{aligned} t \equiv 0 & \begin{cases} p = 2r + 1, & r \equiv 0, 3; \\ p = 2r - 1, & r \equiv 2, 3; \\ p = 2r + 2, & r \equiv 3; \\ p = 2r, & r \equiv 1, 2, 3; \end{cases} \\ t \equiv 1 & \begin{cases} p = 2r + 1, & r \equiv 0; \\ p = 2r - 1, & r \equiv 2, 3, 0; \\ p = 2r - 2, & r \equiv 0; \\ p = 2r, & r \equiv 1, 2, 0; \end{cases} \\ t \equiv 2 & \begin{cases} p = 2r - 1, \\ p = 2r, \end{cases} \text{ every } r; \\ t \equiv 3 & \begin{cases} p = 2r + 1, & r \equiv 3; \\ p = 2r - 1, & r \equiv 1, 2, 3; \\ p = 2r - 2, & r \equiv 2, 0; \\ p = 2r, & r \equiv 2, 0 \end{cases} \end{aligned}$$

But, by the nature of the problem, we see easily that, for a given  $n$ , if a value of  $p$  is possible, a smaller value of  $p$  is also possible. Guided by this principle we choose, for a given  $r$ , the largest value  $p_1$  of  $p$  in the above table, obtaining

$$\begin{array}{l}
 t \equiv 0 \left\{ \begin{array}{ll} r = 4\alpha + 0: & p_1 = 2r + 1 = 8\alpha + 1; \\ r = 4\alpha + 1: & p_1 = 2r = 8\alpha + 2; \\ r = 4\alpha + 2: & p_1 = 2r = 8\alpha + 4; \\ r = 4\alpha + 3: & p_1 = 2r + 2 = 8\alpha + 8; \end{array} \right. \\
 t \equiv 1 \left\{ \begin{array}{ll} r = 4\alpha + 0: & p_1 = 2r + 1 = 8\alpha + 1; \\ r = 4\alpha + 1: & p_1 = 2r = 8\alpha + 2; \\ r = 4\alpha + 2: & p_1 = 2r = 8\alpha + 4; \\ r = 4\alpha + 3: & p_1 = 2r - 1 = 8\alpha + 5; \end{array} \right. \\
 t \equiv 2 \left\{ \begin{array}{ll} r = 4\alpha + 0: & p_1 = 2r = 8\alpha + 0; \\ r = 4\alpha + 1: & p_1 = 2r = 8\alpha + 2; \\ r = 4\alpha + 2: & p_1 = 2r = 8\alpha + 4; \\ r = 4\alpha + 3: & p_1 = 2r = 8\alpha + 6; \end{array} \right. \\
 t \equiv 3 \left\{ \begin{array}{ll} r = 4\alpha + 0: & p_1 = 2r = 8\alpha + 0; \\ r = 4\alpha + 1: & p_1 = 2r - 1 = 8\alpha + 1; \\ r = 4\alpha + 2: & p_1 = 2r = 8\alpha + 4; \\ r = 4\alpha + 3: & p_1 = 2r + 1 = 8\alpha + 7. \end{array} \right.
 \end{array}$$

But if we write a given  $n$  in the form  $\mu \cdot 2^r$ ,  $r$  is greatest when  $\mu$  is odd. This furnishes (by the above table) the maximum possible values  $p_1$  of  $p$  in the various cases. Theorem 2 is proved.

As a consequence of Theorem 2 let us remark that in the complex domain the distinction between  $t \equiv 0, 1, 2, 3 \pmod{4}$  disappears,  $g(u)$  being reducible to a sum of squares. In this case, of the four sets of values  $p_1$  given in the above table, we should take the first set which has largest values. Hence, in the complex, we have simply  $p \leq 8\alpha + 2^6$ . This is precisely Hurwitz' classical theorem in Radon's form.<sup>4</sup> For positive definite forms it was first shown by Radon that this Hurwitz theorem holds also in the real.<sup>5</sup>

<sup>1</sup> Dubisch, R., *Ann. of Math.*, 47, 510-527 (1946).

<sup>2</sup> Hurwitz, A., *Math. Ann.*, 88, 1 (1923).

<sup>3</sup> Dubisch, *loc. cit.*, 520.

<sup>4</sup> Radon, J., *Abh. Sem. Hamburg*, 1, 1-25 (1922).

<sup>5</sup> See also Eckmann, B., *Comment. Math. Helv.*, 15, 358-366 (1943).



## ON THE PROBLEM OF SIMILAR REGIONS

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Fundamental in the Neyman-Pearson theory of testing composite statistical hypotheses is the problem of similar regions, that is, the problem of determining the totality of similar regions. In general a point set  $w$  in a space  $W$  might be defined as a similar region with respect to a family of measures on  $W$  if every measure in the family assigns the same value to  $w$ . In the case treated here  $W$  is a Euclidean space of points  $x = (x_1, \dots, x_n)$ , and the family of measures is generated by a probability density<sup>1</sup>  $p(x; \theta)$  depending on a parameter  $\theta = (\theta_1, \dots, \theta_s)$ . The family of measures is then represented by the set  $\omega$  of permissible parameter points  $\theta$ , the measure of a set  $w$  being

$$\int_w p(x; \theta) dx. \quad (1)$$

We make the blanket assumptions that all point sets considered are Borel sets, and all functions are Borel-measurable.

The problem of similar regions was introduced by Neyman and Pearson<sup>2</sup> and solved for certain cases. These results were later generalized by Neyman<sup>3</sup> under the assumptions that the probability density satisfied certain partial differential equations (equations (6) below) and that a certain moment problem had a unique solution; this development was continued by Scheffé.<sup>4</sup> The problem was approached very differently by P. L. Hsu;<sup>5</sup> he utilized the uniqueness theorem of the theory of Laplace transforms. The latter method was extended by Lehmann<sup>6</sup> and is still further generalized in the present paper. Under this approach no moment problem intrudes itself, and cases are included which do not yield to the differential equations attack. We are concerned here only with the problem of similar regions; we shall present in a later paper applications to the theory of optimum tests, which gives the problem of similar regions its statistical interest. We outline here without the proofs results which will later be published elsewhere at length.

Our development is based on the notion of a *complete kernel*. Consider a non-negative function  $g(t; \theta)$ , where  $t = (t_1, \dots, t_s)$  is a point in an  $s$ -dimensional Euclidean space. If for some  $\theta$  in  $\omega$  the function  $g(t; \theta)$  is not defined over the whole  $t$ -space its definition is to be extended by assigning it the value zero. With the function  $g(t; \theta)$  we associate a set  $S$  in the  $t$ -space defined as follows: For each  $\theta$  in  $\omega$  we let  $N_\theta$  be the set of points  $t_0$  for which there exists a neighborhood of  $t_0$  such that the integral

of  $g(t; \theta)$  over the neighborhood vanishes. The set  $S$  is the complement of the intersection of the  $N_\theta$  for  $\theta$  in  $\omega$ . Then we shall call  $g(t; \theta)$  a complete kernel with respect to  $\omega$  if the two conditions

$$(i) \quad \int f(t)g(t; \theta)dt = 0$$

for all  $\theta$  in  $\omega$ , and (ii)  $f(t)$  is bounded, imply that  $f(t) = 0$  for almost all  $t$  in  $S$ . It is understood that when a domain of integration is not indicated, as in (i) above, then it is the whole space of the variable of integration.

It is convenient at this point to employ more of the terminology of the theory of probability. Let  $X = (X_1, \dots, X_n)$  be a random variable with the probability density  $p(x; \theta)$ , so that the probability that  $X$  fall in any set  $w$  is given by (1). Suppose now that there exist real-valued functions  $h(x)$  and  $q(t; \theta)$ , and a vector-valued function  $t(x) = (t_1(x), \dots, t_s(x))$  with  $s$  components, such that for all  $\theta$  in  $\omega$  and  $x$  in  $W$  the density has the form

$$p(x; \theta) = q(t; \theta)h(x), \quad (2)$$

where  $t = t(x)$  and  $s < n$ . Let  $G$  be the set of points in the  $x$ -space for which  $h(x) \neq 0$ . It will suffice to assume that  $t(x)$  is defined for all  $x$  in  $G$ , and that  $q(t; \theta)$  is defined for all  $\theta$  in  $\omega$  and all  $t$ -values assumed by  $t(x)$  for  $x$  in  $G$ .

Consider the statistic  $T = (T_1, \dots, T_s)$ , where  $T = t(X)$ : If in  $G$  the vector function  $t = t(x)$  satisfies certain regularity conditions (namely, (a), (b), (c) of Lemma 1 with  $r = s$ ) then  $T$  has a probability density for all  $\theta$  in  $\omega$ . There then exists a function  $\psi(t)$  such that the probability density of  $T$  is

$$g(t; \theta) = q(t; \theta)\psi(t). \quad (3)$$

$T$  is a sufficient statistic for  $\theta$ : This means that for any set  $w$  in  $W$  the conditional probability that  $X$  fall in  $w$ , given that  $T = t$ , is independent of  $\theta$ .

Neyman<sup>7</sup> pointed out that when there exists a sufficient statistic  $T$  for  $\theta$ , then a sufficient condition for a set  $w$  to be a similar region (of fixed probability  $\alpha$ ) is that the intersections  $w(t)$  of  $w$  with the "surface"  $T = t$  have the property that the conditional probability of  $w$ , given  $T = t$ , be equal to  $\alpha$  for almost all  $t$  in  $S$ . He left open the question whether all similar regions necessarily have this structure, which we shall call the *Neyman structure* with respect to the statistic  $T$ . This question is settled by Theorem 1 below. In the proof of Theorem 1 and Corollary 1 we apply

**LEMMA 1.** *Suppose  $r$  real-valued functions  $t_1(x), \dots, t_r(x)$  of the point  $x = (x_1, \dots, x_n)$  are defined on a set  $G$ , and that the following conditions are satisfied:*

$$(a) \quad r < n.$$

(b) The boundary of  $G$  is a set of (Lebesgue) measure zero.

(c) The  $r$  functions  $t_i(x)$  together with their first partial derivatives are continuous and the  $r \times n$  matrix  $(\partial t_i / \partial x_j)$  is of rank  $r$ , except in a subset  $N$  of  $G$ , whose closure has measure zero.

Then there exist  $n-r$  functions  $t_{r+1}(x), \dots, t_n(x)$  defined in an open subset  $G^*$  of  $G$ , such that the following conditions are satisfied:

(A) The closure of the set  $G-G^*$  has measure zero.

(B) The transformation  $y = f(x)$ , where  $y = (y_1, \dots, y_n)$  and  $f(x) = (t_1(x), \dots, t_n(x))$ , is 1:1 and bicontinuous from  $G^*$  to  $f(G^*)$ .

(C) The  $n$  functions  $t_1(x), \dots, t_n(x)$  together with their first partial derivatives are continuous and the Jacobian

$$J(x) = \partial(y_1, \dots, y_n) / \partial(x_1, \dots, x_n)$$

does not vanish in  $G^*$ .

For a transformation  $y = f(x)$  of the kind whose existence is guaranteed by Lemma 1, the usual transformation of multiple integrals by means of the Jacobian is valid: Specifically, if  $w$  is any set in  $G$ , if  $w^*$  is the intersection of  $w$  with  $G^*$ , and if  $p(x)$  is integrable over  $w$ , then

$$\int_w p(x) dx = \int_{w^*} p(x) dx = \int_{f(w^*)} [p(x) / |J(x)|] dy,$$

where in the last integral  $x = f^{-1}(y)$ .

**THEOREM 1.** If the probability density  $p(x; \theta)$  has the form (2), and if the function  $t_1(x), \dots, t_r(x)$  satisfy conditions (a), (b), (c) of Lemma 1 with  $r = s$ , so that  $T = t(X)$  is a sufficient statistic for  $\theta$  with a probability density  $g(t; \theta)$  of the form (3), then a necessary and sufficient condition for the totality of similar regions with respect to  $\theta$  to have the Neyman structure with respect to  $T$  is that the density  $g(t; \theta)$  be a complete kernel with respect to  $\omega$ .

The following corollary to Theorem 1 includes as special cases all instances in which the problem of similar regions has previously been solved. We recall that  $s$  and  $n$  denote the number of coordinates of  $\theta$  and  $x$ , respectively.

**COROLLARY 1.** Suppose there exist functions  $C, h, k_i, t_i$  ( $i = 1, \dots, r$ ), and constants  $c_i$  ( $i = r' + 1, \dots, r$ ), where  $0 \leq r' \leq r \leq s$ , such that for all  $\theta$  in  $\omega$

$$p(x; \theta) = C(\theta)h(x)\exp. \left\{ \sum_{i=1}^{r'} k_i(\theta)t_i(x) \right\} \quad (4)$$

in the part of the  $x$ -space determined by the inequalities

$$c_i < t_i(x) < k_i(\theta) \quad (i = r' + 1, \dots, r), \quad (5)$$

and  $p(x; \theta)$  vanishes elsewhere, and that the following conditions are satisfied:

(a) In  $G$ , defined to be the part of the  $x$ -space where  $h(x) \neq 0$ , the functions  $t_1(x), \dots, t_r(x)$  satisfy the conditions (a), (b), (c) of Lemma 1.

(b) The set of values assumed by  $(k_1, \dots, k_r)$  is the Cartesian product of the set  $L_1$  of values assumed by  $(k_1, \dots, k_{r'})$  and the set  $L_2$  of values assumed by  $(k_{r'+1}, \dots, k_r)$ . The set  $L_1$  contains a non-degenerate  $r'$ -dimensional interval. The set  $L_2$  is an  $(r - r')$ -dimensional interval  $c_i < k_i < d_i$  ( $i = r' + 1, \dots, r$ ), where the  $c_i$  are the same as in (5), and where the  $c_i$  and  $d_i$  may be infinite.

Then the totality of similar regions with respect to  $\theta$  has the Neyman structure with respect to the statistic  $T = (T_1, \dots, T_r)$ , where  $T_i = t_i(X)$ .

Neyman<sup>8</sup> assumed in his solution of the problem of similar regions that the probability density satisfied certain conditions including the following partial differential equations:

$$\partial^2 v / \partial \theta_i \partial \theta_j = A_{ij}(\theta) + \sum_{k=1}^s B_{ijk}(\theta) \partial v / \partial \theta_k \quad (i, j = 1, \dots, s), \quad (6)$$

where  $v(x; \theta) \equiv \log p(x; \theta)$ . That a density satisfying Neyman's conditions is included under the special case of our Corollary 1 when  $r' = r$  follows from

**THEOREM 2.** Suppose the probability density  $p(x; \theta)$  satisfies the following conditions:

- (a) The set  $\omega$  of permissible parameter points  $\theta$  is a connected open set.
- (b) The set  $W_+ = W_+(\theta)$  of points in the  $x$ -space for which  $p(x; \theta) > 0$  does not depend on  $\theta$ .
- (c) For all  $\theta$  in  $\omega$  and  $x$  in  $W_+$ ,  $p(x; \theta)$  and its first and second partial derivatives with respect to  $\theta_1, \dots, \theta_s$  are continuous function of  $\theta$ .
- (d) There exist continuous functions  $A_{ij}(\theta)$  and  $B_{ijk}(\theta)$  ( $i, j, k = 1, \dots, s$ ) such that  $v = \log p(x; \theta)$  satisfies the system (6) of partial differential equations for all  $\theta$  in  $\omega$  and  $x$  in  $W_+$ .

Then there exist an integer  $r$  ( $0 \leq r \leq s$ ), real-valued functions  $C, h, t_i, k_i$  ( $i = 1, \dots, r$ ) such that

(A) The density  $p(x; \theta)$  has the form (4) with  $r' = r$  for all  $\theta$  in  $\omega$  and  $x$  in  $W_+$ .

(B) The  $r + 1$  functions  $1, t_1, \dots, t_r$  are linearly independent for  $x$  in  $W_+$ .

(C) The  $r + 1$  functions  $C, k_1, \dots, k_r$  together with their first and second partial derivatives with respect to  $\theta_1, \dots, \theta_s$  are continuous, while the  $r \times s$  matrix  $(\partial k_i / \partial \theta_j)$  is of rank  $r$ , for all  $\theta$  in  $\omega$ .

If the  $r$  of Theorem 2 is zero we have the trivial case where  $p(x; \theta)$  does not depend on  $\theta$ , and all sets  $w$  are similar regions. If  $r > 0$ , result (C) of Theorem 2 insures condition (b) of Corollary 1 with  $r' = r$ . Condition (a) of Corollary 1 on the functions  $t_i = t_i(x)$  cannot be implied by the differential equations (6) which govern the behavior of  $p(x; \theta)$  as a function of  $\theta$ . However, if we define the set  $G$  of Corollary 1 as the  $W_+$  of Theorem 2 and add to the hypotheses of Theorem 2 the assumption that in  $G$  the functions  $t_i(x)$  of Theorem 2 satisfy conditions (a), (b), (c) of Lemma 1

(a similar assumption was made by Neyman), then all the hypotheses for the special case of Corollary 1 when  $r' = r$  are satisfied.

The converse to Theorem 2, that if the density has the form (4) with  $r' = r$ , then it satisfies a system of partial differential equations of the form (6), is easily proved under mild restrictions by differentiating (4).

<sup>1</sup> By probability density we mean, as usual, any non-negative function whose integral over  $W$  is unity.

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<sup>4</sup> Scheffé, H., *Ibid.*, 13, 280-293 (1942).

<sup>5</sup> Hsu, P. L., *Biometrika*, 32, 62-69 (1941).

<sup>6</sup> Lehmann, E. L., *Annals of Math. Stat.*, in press.

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## A PROOF OF LOWER SEMICONTINUITY

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McShane has a pair of equivalent theorems<sup>1</sup> affirming the lower semicontinuity of simple integrals of the calculus of variations in the parametric and non-parametric form. The hypotheses are broad enough to permit the restrictions on the domain of definition associated with Lagrange problems. In this paper we shall give a brief direct proof of the theorem in the parametric form and comment on an apparent generalization of the theorem. We shall discuss in turn the domain of definition, the auxiliary function, curves, the integral, the theorem with proof and the generalization.

Before proceeding we wish to express our thanks to Professor Transue for reading critically both this paper and an earlier and longer draft and for encouraging us to make one more attempt at this particular form of proof.

The domain  $R$  is a subset of the space of points  $(x, r) = (x^1, \dots, x^n, r^1, \dots, r^n)$  subject to two restrictions.

R1. If  $(x, r) \in R$  and  $k \geq 0$  then  $(x, kr) \in R$ .

R2. The set  $R_0$  of points  $(x, 0) \in R$  is closed.<sup>2</sup>

The auxiliary function  $f(x, r)$  is a function satisfying the following requirements.

f1.  $f(x, r)$  is a real valued function defined on a domain  $R$  satisfying R1, 2 and admits  $+\infty$  (but not  $-\infty$ ) as a value.



f2.  $f(x, kr) = kf(x, r)$  if  $(x, r) \in R$  and  $k \geq 0$ .  $f(x, 0) = 0$  if  $(x, 0) \in R$ .

f3. If  $(x_0, r_0) \in R$  and  $f(x_0, r_0) > u$  then there is a vector  $a$  such that  $a \cdot r_0 > u$  and such that corresponding to  $\epsilon > 0$  there is a  $\delta > 0$  for which  $f(x, r) \geq a \cdot r - \epsilon|r|$  whenever  $|x - x_0| < \delta$  and  $(x, r) \in R$ .

It follows readily that  $f$  is lower semicontinuous and that  $f$  is convex in  $r$  in the sense that if  $(x_0, r_0) \in R$  and  $f(x_0, r_0) > u$  then there is a vector  $a$  such that  $a \cdot r_0 > u$  and  $f(x_0, r) \geq a \cdot r$  if  $(x_0, r) \in R$ . As an approximate converse, if  $f(x, r)$  satisfies f1, 2 and is lower semicontinuous and convex in  $r$  and if the domain  $R$  is closed then  $f$  satisfies f3.

We recall that parameterized curves  $x = x(t)$ ,  $t_1 \leq t \leq t_2$ , can be classified, two parameterized curves being regarded as equivalent if their Fréchet distance is 0. An equivalence class is called a curve. Then in the usual way curves are a metric space with Fréchet metric as the distance. A parameterized curve is admissible if the coördinate functions are absolutely continuous and if  $(x(t), x'(t)) \in R$  for almost all  $t$ . As a special convention, a curve consisting of a single point is admissible only if the point is in  $R$ . If any parameterized curve in a class is admissible then all absolutely continuous parameterized curves in the class, including the one with arc length as parameter, are admissible and the curve is called admissible.

The integral

$$\int_{t_1}^{t_2} f[x(t), x'(t)] dt \quad (1)$$

is evaluated for admissible parameterized curves, the integrand being measurable, and its value is the same for all those in the same curve class. Thus the integral (1) defines a function  $J(C)$  of admissible curves  $C$ . In this connection we extend the usual definition of the Lebesgue integral to admit  $+\infty$  as a value if that is the limit of the integral of the truncated function. This will not present the difficulties sometimes inherent in extending the Lebesgue integral because in this extension the negative part of the function will remain bounded by a function integrable in the usual sense. This point is discussed more fully in the course of the proof of the principal theorem.

This leads to the following theorem.

**THEOREM (McSHANE).** *If the auxiliary function  $f(x, r)$  satisfies f1, 2, 3 then the function  $J(C)$  of admissible curves is lower semicontinuous on any set of admissible curves of bounded length.*

The proof by McShane consists of approximating  $f$  from below by smoother functions for which the corresponding integrals are more readily proved to be lower semicontinuous. The proof to be given here will consist of passage from the inequalities of f3, applied along the limiting curve, to the inequality of lower semicontinuity almost directly by integration.



We are to prove that if  $C_q \rightarrow \bar{C}$  in the Fréchet metric and  $C_q$  and  $\bar{C}$  are admissible then

$$\lim_{q \rightarrow \infty} J(C_q) \geq J(\bar{C}). \quad (2)$$

The following preliminaries to the proof are familiar and are essentially those used by McShane.

*It is sufficient to assume  $\lim_{q \rightarrow \infty} J(C_q)$  exists (finite or infinite) since every inferior limit is a limit. It is sufficient to assume  $J(C_q)$  finite for all  $q$ , since if  $J(C_q) = +\infty$  for an infinite number of values of  $q$  then  $\lim_{q \rightarrow \infty} J(C_q) = +\infty$  and (2) holds.*

Curves  $C_q$  are parameterized on the interval  $0 \leq t \leq 1$  by the parameter proportional to arc length (this can be so worded as to include the unique parameterization of curves of 0 length). Let  $x_q(t)$  thus represent  $C_q$ ; this is an admissible parameterization. Because of the bounded length and the Fréchet convergence there is a constant  $M$  such that

$$|x_q(t)| \leq M, \quad (3)$$

$$|x_q'(t)| \leq M \quad (4)$$

where it exists and

$$\left| \frac{x_q(t'') - x_q(t')}{t'' - t'} \right| \leq M \quad 0 \leq t' < t'' \leq 1. \quad (5)$$

From the sequence  $x_q(t)$  a uniformly convergent subsequence, still denoted by  $x_q(t)$  to avoid double subscripts, is selected<sup>4</sup> with limit  $\bar{x}(t)$ , for which then

$$|\bar{x}(t)| \leq M, \quad (6)$$

$$|\bar{x}'(t)| \leq M \quad (7)$$

where it exists and

$$\left| \frac{\bar{x}(t'') - \bar{x}(t')}{t'' - t'} \right| \leq M \quad 0 \leq t' < t'' \leq 1. \quad (8)$$

On the one hand  $C_q$  approaches  $\bar{C}$  and on the other hand it approaches the curve<sup>6</sup> represented by  $\bar{x}(t)$ . Thus  $x = \bar{x}(t)$  is an admissible parameterization of  $\bar{C}$ .

We adopt the following abbreviations.

$$f_q = f_q(t) = f[x_q(t), x_q'(t)] \quad \bar{f} = \bar{f}(t) = f[\bar{x}(t), \bar{x}'(t)]. \quad (9)$$

We observe that *there is a number  $N$  such that for all  $q$  (after discarding a finite number for convenience if necessary) and almost all  $t$*

$$f_q(t) \geq -Nx_q'(t) \quad \bar{f}(t) \geq -N\bar{x}'(t). \quad (10)$$

For with  $u = -1$ ,  $\epsilon = 1$ , we apply<sup>6</sup>  $f3$  at  $(\bar{x}(t), 0)$  to obtain a vector  $a(t)$  and a number  $\delta(t) > 0$  for which

$$f(x, r) \geq a(t) \cdot r - |r| \quad (11)$$

whenever

$$|x - \bar{x}(t)| < \delta(t).$$

Taking a finite covering of the curve  $\bar{C}$  by spheres with center  $(\bar{x}(t), 0)$  and radius  $\delta(t)$  and letting  $N - 1$  denote the maximum of the corresponding values of  $|a(t)|$  one sees that

$$f(x, r) \geq -N|r| \quad (12)$$

whenever  $x$  belongs to a suitable open set containing  $\bar{C}$  and  $(x, r) \in R$ . Excluding values of  $t$  for which  $x_q'(t)$  or  $\bar{x}'(t)$  fail to exist or  $(x_q(t), x_q'(t))$  or  $(\bar{x}(t), \bar{x}'(t))$  fail to be in  $R$  and excluding a finite number of values of  $q$  as noted, one sees the truth of (10).

If  $M_0 = NM$ , then almost everywhere

$$f_q \geq -M_0 \quad \bar{f} \geq -M_0. \quad (13)$$

Relation (12) or more particularly (13) implies that truncation above is adequate for the definition of integrals of unbounded functions and that the trivial extension of Lebesgue integration which we have made does not introduce conditionally convergent integrals.

To complete the proof we have only to establish the following statement.

STATEMENT A. Corresponding to numbers  $\eta > 0$  and  $U < \int_0^1 \bar{f} dt$  there is a set  $E$  of measure exceeding  $1 - \eta$  such that

$$\lim_{q \rightarrow \infty} \int_E f_q dt \geq U. \quad (14)$$

For

$$\int_0^1 f_q dt \geq \int_E f_q dt - M_0 \eta \quad (15)$$

by virtue of (13) so that

$$\lim_{q \rightarrow \infty} \int_0^1 f_q dt \geq U - M_0 \eta \quad (16)$$

for all  $\eta$ ,  $U$  conditioned as in Statement A, whence

$$\lim_{q \rightarrow \infty} \int_0^1 f_q dt \geq \int_0^1 \bar{f} dt \quad (17)$$

and (2) and the theorem follow.

We shall now prove Statement A in several steps. There is a set  $E_0$

of measure 1 on the unit interval on which  $\bar{x}'(t)$  and all  $x_q'(t)$  are defined and  $(\bar{x}(t), \bar{x}'(t))$  and  $(x_q(t), x_q'(t))$  are in  $R$ . For  $t \in E_0$ , let

$$u(t) = \min f(t) - \epsilon', Q \quad (18)$$

where  $\epsilon' > 0$  is so small and  $Q > 0$  is so large that

$$\int_0^1 u(t) dt > U. \quad (19)$$

By virtue of a theorem of Lusin<sup>7</sup> there is a closed subset  $E$  of  $E_0$  with measure exceeding  $1 - \eta$  such that when their domain of definition is restricted to  $E$ ,  $\bar{x}'(t)$  and  $u(t)$  are continuous functions. Taking advantage of the absolute continuity of the integral, one also requires that

$$\int_E u(t) dt > U. \quad (20)$$

This will be shown to be the set  $E$  required for Statement A.

As a further step toward the proof of Statement A we shall establish that *corresponding to  $\epsilon > 0$  there are a bounded measurable vector function  $b(t)$  defined on  $E$  and a number  $h > 0$  such that*

$$b(t) \cdot \bar{x}'(t) > u(t) \quad (21)$$

$$f(x, r) \geq b(t) \cdot r - \epsilon|r| \quad \text{if } |x - \bar{x}(t)| < h \text{ and } (x, r) \in R. \quad (22)$$

For each point  $t \in E$  with  $u = u(t)$  there is a vector  $a = a(t)$  and a number  $\delta = \delta(t)$  meeting the requirements

$$a(t) \cdot \bar{x}'(t) > u(t) \quad (23)$$

$$f(x, r) \geq a(t) \cdot r - \epsilon|r| \quad \text{if } |x - \bar{x}(t)| < \delta(t) \text{ and } (x, r) \in R$$

by virtue of  $f_3$  applied at points  $(\bar{x}(t), \bar{x}'(t))$ . (However there is no reason to suppose  $a(t)$  is bounded and measurable.) Then for each point  $t \in E$  there is a number  $\theta(t) > 0$  such that if  $|t - t^*| < \theta(t)$  and  $t^* \in E$  then

$$a(t) \cdot \bar{x}'(t^*) > u(t^*) \quad (24)$$

$$|\bar{x}(t^*) - \bar{x}(t)| < \delta(t)/2, \quad (25)$$

these statements depending on the continuity of  $\bar{x}'(t)$ ,  $u(t)$ ,  $\bar{x}(t)$ . We use the Heine-Borel theorem on the covering of  $E$  by intervals  $t - \theta(t)$ ,  $t + \theta(t)$ ; we denote by  $t_j$ ,  $j = 1, \dots, p$ , the points  $t$  centered in the elements of the finite covering and by  $I_j$  the part of  $E$  covered by the interval  $t_j - \theta(t_j)$ ,  $t_j + \theta(t_j)$ . Let  $b(t) = a(t_1)$  for  $t \in I_1$  and let  $b(t) = a(t_j)$  for  $t \in I_j$  and  $t \notin I_1 \cup \dots \cup I_{j-1}$ . Then  $b(t)$  is bounded and measurable. Let  $h = \min \delta(t_j)/2$ . Then suppose  $t^* \in I_j$  and  $t^* \notin I_1 \cup \dots \cup I_{j-1}$  (empty if  $j = 1$ ). Then  $|t_j - t^*| < \theta(t_j)$  so that

$$b(t^*) \cdot \bar{x}'(t^*) = a(t_j) \cdot \bar{x}'(t^*) > u(t^*) \quad (26)$$

by virtue of (24). Also if  $|x - \bar{x}(t^*)| < h \leq \delta(t_j)/2$ , then, using (25) with  $t = t_j$ ,  $|x - \bar{x}(t_j)| < \delta(t_j)$  and

$$f(x, r) \geq a(t_j) \cdot r - \epsilon|r| = b(t^*) \cdot r - \epsilon|r| \text{ if } (x, r) \in R. \quad (27)$$

Thus the existence of  $b(t)$  is proved.

To complete the proof of Statement A, we observe, following (22) and (4), that

$$\int_E f_q dt \geq \int_E b(t) \cdot x_q'(t) dt - \epsilon \int_E |x_q'(t)| dt \geq \int_E b(t) \cdot x_q'(t) dt - \epsilon M \quad (28)$$

if  $q$  is large enough that  $|x_q(t) - \bar{x}(t)| < h$ . But

$$\lim_{q \rightarrow \infty} \int_E b(t) \cdot x_q'(t) dt = \int_E b(t) \cdot \bar{x}'(t) dt \quad (29)$$

by virtue of a theorem of Lebesgue.<sup>8</sup> Thus, following (21) and (20),

$$\lim_{q \rightarrow \infty} \int_E f_q dt \geq \int_E b(t) \cdot \bar{x}'(t) dt - \epsilon M > \int_E u(t) dt - \epsilon M > U - \epsilon M \quad (30)$$

for all  $\epsilon > 0$ , whence Statement A and the theorem.

We have a generalization whose import we do not fully understand. Hypothesis  $f3$  contains a statement of uniformity in that vectors  $a$  are hypothesized which are fixed while  $\epsilon$  may change. We find no place, either in the preliminaries of admissibility of curves and of the fact that the value of the integral is independent of admissible parameterization or in the proof itself, where this uniformity is used. We find instead that *the theorem still holds when  $f3$  is replaced by the following hypothesis.*

$f3a$ . If  $(x_0, r_0) \in R$  and  $f(x_0, r_0) > u$  then corresponding to  $\epsilon > 0$  there is a vector  $a$  such that  $a \cdot r_0 > u$  and a number  $\delta > 0$  for which  $f(x, r) \geq a \cdot r - \epsilon|r|$  whenever  $|x - x_0| < \delta$  and  $(x, r) \in R$ .

It is obvious that  $f3$  implies  $f3a$  but whether  $f3a$  implies  $f3$  in the presence of  $f1, 2$  is not known to the author. It follows from  $f1, 2, 3a$  that  $f(x, r)$  is lower semicontinuous. It does not follow obviously for the writer that  $f(x, r)$  is a convex function of  $r$  for  $x$  fixed, in the sense that the word convex has been used earlier. (If this were true then  $f3a$  would be equivalent to  $f3$  in the presence of  $f1, 2$  for closed  $R$  though not necessarily for all  $R$  satisfying  $R1, 2$ .) It does follow that  $f(x, r)$  is a convex function if additional hypotheses, such as that  $a$  can be found in  $f3a$  bounded independent of  $\epsilon$  (or that  $f(x, r)$  is defined for all  $r$  and bounded for unit vectors  $r$ ), are imposed.

The utility of the generalization at the moment is the fact that  $f3a$  may be easier to verify than  $f3$  in an application.

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<sup>1</sup> McShane, E. J., "Semi-Continuity of Integrals in the Calculus of Variations," *Duke Math. J.*, 2, 597-616 (1936). See Theorems 3.1, 4.1, of which we are concerned with the former.

<sup>2</sup> We have been unable to see the necessity of McShane's assumption that  $R_0$  be dense in itself. We admit the triviality of isolated points of  $R_0$ .

<sup>3</sup>  $a \cdot r$  denotes the scalar product and  $|r| = \sqrt{r \cdot r}$ .

<sup>4</sup> By virtue of Ascoli's theorem applied successively to coördinate functions. See Kellogg, O. D., *Foundations of Potential Theory*, Springer, 1929, p. 265.

<sup>5</sup> The Fréchet distance between  $x_0(t)$  and  $x(t)$  does not exceed  $\max |x_0(t) - x(t)|$ .

<sup>6</sup> The point  $(x(t), x'(t))$  is in  $R$  for almost all  $t$ . Thus, by virtue of  $R_1$ , so is  $(x(t), 0)$ . Thus, because of  $R_2$ , the point  $(x(t), 0)$  is in  $R$  for all  $t$ .

<sup>7</sup> See for easy reference Saks, S., "Theory of the Integral," *Monografie Matematyczne*, VII, Warszawa-Lwow, 1937, p. 72 or McShane, E. J., "Integration," *Princeton Mathematical Series*, 7, Princeton, 1944, p. 236.

<sup>8</sup> Convenient statement of the theorem in Banach, S., "Théorie des opérations linéaires," *Monografie Matematyczne*, I, Warszawa, 1932, p. 7. Original reference is Lebesgue, H., "Sur les intégrales singulières," *Annales de Toulouse* 3<sup>e</sup> série, 1 (1909), 25-117; see p. 57. Other convergence theorems also cover this point.

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